PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 23 May 2001 (23.05.01)	
International application No. PCT/EP00/08554	**Applicant's or agent's file reference** 002219woMebk
International filing date (day/month/year) 01 September 2000 (01.09.00)	**Priority date** (day/month/year) 01 September 1999 (01.09.99)
Applicant RICHARD, Giesbert et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   10 March 2001 (10.03.01)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Olivia TEFY
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
002219wo Me/sh		

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/08554	01/09/2000	01/09/1999

International Patent Classification (IPC) or national classification and IPC
C12Q1/68

Applicant
EVOTEC NEUROSCIENCES GMBH et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10/03/2001	02.07.2001

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer

Goetz, M

Telephone No. +49 89 2399 8697

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/08554

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-42 as originally filed

Claims, No.:

1-40 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 29-33, 34-40.

because:

☒ the said international application, or the said claims Nos. 29-33 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 34-40.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-33.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-33
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-33
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-28
	No:	Claims	

2. Citations and explanations
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 29 - 33 relate to an in vivo therapeutic treatment of the human/animal body; hence, they relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT), see section V below.

However, an opinion with respect to novelty / inventive step will be expressed on the basis of the alleged technical effects recited in the said claims.

Re Item IV

Lack of unity of invention

1. The IPEA agrees with the objection already put forward by the ISA as to lack of unity (Rule 13 PCT), the reasons for the objection being as follows:

Claims 1 - 33 recite methods relating to the diagnosis, the monitoring, the treatment or the evaluation of such treatment of age-related macular degeneration, as well as a the use of a kit for the diagnosis or prognosis of increased risk of age-related macular degeneration, said methods and kit being based on the determination of activity/level/mutations/polymorphisms of Cystatin C, its transcription/translation products and/or amyloid protein.

Claims 34 - 40 recite modulators which affect the activity and/or level of Cystatin C, its transcription/translation products and/or amyloid protein, method for identifying such modulator, medicament comprising said modulator and first/second medical use thereof.

Being directed to modulators which affect the activity and/or level of Cystatin C, its transcription/translation products and/or amyloid protein, to methods for identifying such modulator, as well as to medicaments comprising said modulator and

first/second medical uses thereof, claims 34 - 40 have no common technical link to claims 1 - 33, wherein the technical contribution resides in the function of Cystatin C, its transcription/translation products and/or amyloid protein as markers for age-related macular degeneration.

Re Item V

Reasoned statement under Art. 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The following documents are referred to in this Report:

   D1: US-A-5 231 000
   D2: EP-A-0 391 714
   D3: US-A-5 270 165

2. None of the available documents attempts to connect levels or activities of Cystatin C or amyloid proteins with the occurrence of age-related macular disease.

   Claims 1 - 24 and 29 - 33, directed to methods for the diagnosis/prognosis, the monitoring or the evaluation of a treatment of age-related macular degeneration, said methods relying on this newly observed difference of said levels or activities of Cystatin C or amyloid proteins between healthy patients and patients affected with age-related macular disease, are thus novel and involve an inventive step; the said claims therefore meet the requirements according to Art. 33(2) and (3) PCT.

3. According to the present description (see e.g. pages 10 and 11), a reagent which selectively reacts with or detects amyloid protein (or a translation product of an amyloid protein gene) is constituted by a polyclonal or monoclonal antibody specific for amyloid protein.

   D1 discloses monoclonal antibodies against the A4 sequence variant of beta-amyloid protein, amyloid precursor protein and kits containing them (column

2/lines 6 - 36, column 4/lines 45 - 68); **D2** describes monoclonal antibodies against beta-amyloid protein, amyloid precursor protein and kits containing them (see e.g. page 4/lines 38 - 58, page 12/line 50 - page 13/line 42, claims 17 - 23).

Moreover, a known reagent which selectively inhibits and therefore reacts with Cystatin C is its known biological ligand Cathepsin B.

Another reagent which selectively reacts with or detects a translation product of a Cystatin C gene is obviously a Cystatin C specific antibody (see e.g. pages 10 and 11 of the present description); such antibody is disclosed in **D3**, see claims 23 - 25 and column 3/line 55 - column 4/line 7.

However, none of the said documents discloses or suggests the use of such reagents, alone or in a kit, for the diagnosis/prognosis, the risk assessment, the monitoring or the evaluation of treatments of age-related macular degeneration (see the reasoning in paragraph 2. above).

Accordingly, claims 25 - 28, directed to the use of such reagent or kit containing same, are novel and involve an inventive step; the said claims therefore comply with the requirements of Art. 33(2) and (3) PCT.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
002219woMebk		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 08554	01/09/2000	01/09/1999
Applicant		
EVOTEC NEUROSCIENCES GMBH		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 2

   [ ] as suggested by the applicant.

   [X] *because the applicant failed to suggest a figure.*

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/08554

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 29 - 33 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-33

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1 - 33

   Methods relating to the diagnosis, the monitoring, the treatment or the evaluation of such treatment of age-related macular degeneration, as well as a the use of a kit for the diagnosis or prognosis of increased risk of age-related macular degeneration, said methods and kit being based on the determination of activity/level/mutations/polymorphisms of Cystatin C, its transcription/translation products and/or amyloid protein.

2. Claims: 34 - 40

   Modulators which affect the activity and/or level of Cystatin C, its transcription/translation products and/or amyloid protein, method for identifying such modulator, medicament comprising said modulator and first/second medical use thereof.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/08554

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 C07K14/47 C07K14/81 C07K16/18 C07K16/38
C12N9/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, PAJ, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 231 000 A (MAJOCHA RON ET AL) 27 July 1993 (1993-07-27) column 2, line 6-36 column 4, line 45-68 claims 1-4	1-33
A	EP 0 391 714 A (BRIGHAM & WOMENS HOSPITAL) 10 October 1990 (1990-10-10) page 4, line 48 - line 58 claims 17-23; example 1	1-33
A	US 5 270 165 A (CUNNINGHAM DENNIS D ET AL) 14 December 1993 (1993-12-14) column 3, line 55 -column 4, line 7; claims 23,25	1-33

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 2001	12.06.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Goetz, M

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/08554

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ABRAHAMSON M ET AL: "MOLECULAR CLONING AND SEQUENCE ANALYSIS OF CDNA CODING FOR THE PRECURSOR OF THE HUMAN CYSTEINE PROTEINASE INHIBITOR CYSTATIN C" FEBS LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 216, no. 2, 1 June 1987 (1987-06-01), pages 229-233, XP002038720 ISSN: 0014-5793 abstract page 229, right-hand column, last paragraph -page 230, left-hand column, paragraph 1; figure 2 ---	1-33
A	WO 98 34634 A (ENTREMED INC) 13 August 1998 (1998-08-13) page 5, line 1 -page 6, line 4 page 14, line 19 -page 15, line 6 ---	1-33
A	EP 0 330 725 A (GRUENENTHAL CHEMIE) 6 September 1989 (1989-09-06) page 4, line 53 -page 5, line 13; example 3 -----	1-33

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/08554

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5231000 A	27-07-1993	AT 133338 T	15-02-1996
		AU 626845 B	13-08-1992
		AU 3287989 A	01-08-1989
		CA 1339014 A	25-03-1997
		DE 3854944 D	07-03-1996
		EP 0440619 A	14-08-1991
		WO 8906242 A	13-07-1989
EP 0391714 A	10-10-1990	US 5262332 A	16-11-1993
		AU 633312 B	28-01-1993
		AU 5292290 A	11-10-1990
		CA 2013396 A	05-10-1990
		JP 2291965 A	03-12-1990
		NZ 233109 A	23-12-1992
		JP 3206958 A	10-09-1991
US 5270165 A	14-12-1993	EP 0527823 A	24-02-1993
		US 5427931 A	27-06-1995
		WO 9116628 A	31-10-1991
		US 5213962 A	25-05-1993
WO 9834634 A	13-08-1998	US 5981471 A	09-11-1999
		AU 6277198 A	26-08-1998
		EP 0971723 A	19-01-2000
EP 0330725 A	06-09-1989	DE 3724581 A	02-02-1989
		JP 1124389 A	17-05-1989

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF RECEIPT OF RECORD COPY

(PCT Rule 24.2(a))

To:

MEYERS, Hans-Wilhelm
P.O. Box 10 22 41
50462 Cologne
ALLEMAGNE

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
13 November 2000 (13.11.00)	
Applicant's or agent's file reference	**International application No.**
002219woMebk	PCT/EP00/08554

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

EVOTEC NEUROSCIENCES GMBH (for all designated States except US)
RICHARD, Giesbert et al (for US)

International filing date	:	01 September 2000 (01.09.00)
Priority date(s) claimed	:	01 September 1999 (01.09.99) 01 February 2000 (01.02.00)
Date of receipt of the record copy by the International Bureau	:	02 November 2000 (02.11.00)
List of designated Offices	:	

EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
National :JP,US

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- [X] time limits for entry into the national phase
- [X] confirmation of precautionary designations
- [X] requirements regarding priority documents

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:
Dominique DELMAS

Telephone No. (41-22) 338.83.38

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated in the Notification of Receipt of Record Copy (Form PCT/IB/301) by paying national fees and furnishing translations, as prescribed by the applicable national laws.

The time limit for performing these procedural acts is **20 MONTHS** from the priority date or, for those designated States which the applicant elects in a demand for international preliminary examination or in a later election, **30 MONTHS** from the priority date, provided that the election is made before the expiration of 19 months from the priority date. Some designated (or elected) Offices have fixed time limits which expire even later than 20 or 30 months from the priority date. In other Offices an extension of time or grace period, in some cases upon payment of an additional fee, is available.

In addition to these procedural acts, the applicant may also have to comply with other special requirements applicable in certain Offices. **It is the applicant's responsibility** to ensure that the necessary steps to enter the national phase are taken in a timely fashion. Most designated Offices do not issue reminders to applicants in connection with the entry into the national phase.

For detailed information about the procedural acts to be performed to enter the national phase before each designated Office, the applicable time limits and possible extensions of time or grace periods, and any other requirements, see the relevant Chapters of Volume II of the PCT Applicant's Guide. Information about the requirements for filing a demand for international preliminary examination is set out in Chapter IX of Volume I of the PCT Applicant's Guide.

GR and ES became bound by PCT Chapter II on 7 September 1996 and 6 September 1997, respectively, and may, therefore, be elected in a demand or a later election filed on or after 7 September 1996 and 6 September 1997, respectively, regardless of the filing date of the international application. (See second paragraph above.)

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date. If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with an indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity to furnish the priority document within a time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit is the filing date of the earliest application whose priority is claimed.

WO 01/16364
PCT/EP00/08554

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
MEYERS, Hans-Wilhelm
P.O. Box 10 22 41
50462 Cologne
ALLEMAGNE

AvK | Sg | W | Da | Hi | HPJ | MEJ | TW | JH | KB

16. MRZ 2001

Ka K F01 03 07/01 01 02

Date of mailing (day/month/year)	
08 March 2001 (08.03.01)	
Applicant's or agent's file reference 002219woMebk	**IMPORTANT NOTICE**
International application No. PCT/EP00/08554	**International filing date** (day/month/year) 01 September 2000 (01.09.00)
Priority date (day/month/year) 01 September 1999 (01.09.99)	
Applicant EVOTEC NEUROSCIENCES GMBH et al	

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   EP,JP

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   08 March 2001 (08.03.01) under No. WO 01/16364

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

D

PATENT COOPERATION TREATY

PCT

REC'D 30 JUL 2001
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

3

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
002219wo Me/sh		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/08554	01/09/2000	01/09/1999
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant EVOTEC NEUROSCIENCES GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10/03/2001	02.07.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Goetz, M Telephone No. +49 89 2399 8697

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/08554

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-42 as originally filed

Claims, No.:

1-40 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 29-33, 34-40.

because:

   ☒ the said international application, or the said claims Nos. 29-33 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 34-40.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-33.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-33
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-33
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-28
	No:	Claims	

2. Citations and explanations
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 29 - 33 relate to an in vivo therapeutic treatment of the human/animal body; hence, they relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT), see section V below.

However, an opinion with respect to novelty / inventive step will be expressed on the basis of the alleged technical effects recited in the said claims.

Re Item IV

Lack of unity of invention

1. The IPEA agrees with the objection already put forward by the ISA as to lack of unity (Rule 13 PCT), the reasons for the objection being as follows:

   Claims 1 - 33 recite methods relating to the diagnosis, the monitoring, the treatment or the evaluation of such treatment of age-related macular degeneration, as well as a the use of a kit for the diagnosis or prognosis of increased risk of age-related macular degeneration, said methods and kit being based on the determination of activity/level/mutations/polymorphisms of Cystatin C, its transcription/translation products and/or amyloid protein.

   Claims 34 - 40 recite modulators which affect the activity and/or level of Cystatin C, its transcription/translation products and/or amyloid protein, method for identifying such modulator, medicament comprising said modulator and first/second medical use thereof.

   Being directed to modulators which affect the activity and/or level of Cystatin C, its transcription/translation products and/or amyloid protein, to methods for identifying such modulator, as well as to medicaments comprising said modulator and

first/second medical uses thereof, claims 34 - 40 have no common technical link to claims 1 - 33, wherein the technical contribution resides in the function of Cystatin C, its transcription/translation products and/or amyloid protein as markers for age-related macular degeneration.

Re Item V

Reasoned statement under Art. 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The following documents are referred to in this Report:

   D1: US-A-5 231 000
   D2: EP-A-0 391 714
   D3: US-A-5 270 165

2. None of the available documents attempts to connect levels or activities of Cystatin C or amyloid proteins with the occurrence of age-related macular disease.

   Claims 1 - 24 and 29 - 33, directed to methods for the diagnosis/prognosis, the monitoring or the evaluation of a treatment of age-related macular degeneration, said methods relying on this newly observed difference of said levels or activities of Cystatin C or amyloid proteins between healthy patients and patients affected with age-related macular disease, are thus novel and involve an inventive step; the said claims therefore meet the requirements according to Art. 33(2) and (3) PCT.

3. According to the present description (see e.g. pages 10 and 11), a reagent which selectively reacts with or detects amyloid protein (or a translation product of an amyloid protein gene) is constituted by a polyclonal or monoclonal antibody specific for amyloid protein.

   D1 discloses monoclonal antibodies against the A4 sequence variant of beta-amyloid protein, amyloid precursor protein and kits containing them (column

2/lines 6 - 36, column 4/lines 45 - 68); **D2** describes monoclonal antibodies against beta-amyloid protein, amyloid precursor protein and kits containing them (see e.g. page 4/lines 38 - 58, page 12/line 50 - page 13/line 42, claims 17 - 23).

Moreover, a known reagent which selectively inhibits and therefore reacts with Cystatin C is its known biological ligand Cathepsin B.

Another reagent which selectively reacts with or detects a translation product of a Cystatin C gene is obviously a Cystatin C specific antibody (see e.g. pages 10 and 11 of the present description); such antibody is disclosed in **D3**, see claims 23 - 25 and column 3/line 55 - column 4/line 7.

However, none of the said documents discloses or suggests the use of such reagents, alone or in a kit, for the diagnosis/prognosis, the risk assessment, the monitoring or the evaluation of treatments of age-related macular degeneration (see the reasoning in paragraph 2. above).

Accordingly, claims 25 - 28, directed to the use of such reagent or kit containing same, are novel and involve an inventive step; the said claims therefore comply with the requirements of Art. 33(2) and (3) PCT.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/08554

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 C07K14/47 C07K14/81 C07K16/18 C07K16/38 C12N9/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, PAJ, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 231 000 A (MAJOCHA RON ET AL) 27 July 1993 (1993-07-27) column 2, line 6-36 column 4, line 45-68 claims 1-4 ---	1-33
A	EP 0 391 714 A (BRIGHAM & WOMENS HOSPITAL) 10 October 1990 (1990-10-10) page 4, line 48 - line 58 claims 17-23; example 1 ---	1-33
A	US 5 270 165 A (CUNNINGHAM DENNIS D ET AL) 14 December 1993 (1993-12-14) column 3, line 55 -column 4, line 7; claims 23,25 --- -/--	1-33

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 2001	12.06.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Goetz, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/08554

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ABRAHAMSON M ET AL: "MOLECULAR CLONING AND SEQUENCE ANALYSIS OF CDNA CODING FOR THE PRECURSOR OF THE HUMAN CYSTEINE PROTEINASE INHIBITOR CYSTATIN C" FEBS LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 216, no. 2, 1 June 1987 (1987-06-01), pages 229-233, XP002038720 ISSN: 0014-5793 abstract page 229, right-hand column, last paragraph -page 230, left-hand column, paragraph 1; figure 2 ---	1-33
A	WO 98 34634 A (ENTREMED INC) 13 August 1998 (1998-08-13) page 5, line 1 -page 6, line 4 page 14, line 19 -page 15, line 6 ---	1-33
A	EP 0 330 725 A (GRUENENTHAL CHEMIE) 6 September 1989 (1989-09-06) page 4, line 53 -page 5, line 13; example 3 -----	1-33

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/08554

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5231000 A	27-07-1993	AT 133338 T	15-02-1996
		AU 626845 B	13-08-1992
		AU 3287989 A	01-08-1989
		CA 1339014 A	25-03-1997
		DE 3854944 D	07-03-1996
		EP 0440619 A	14-08-1991
		WO 8906242 A	13-07-1989
EP 0391714 A	10-10-1990	US 5262332 A	16-11-1993
		AU 633312 B	28-01-1993
		AU 5292290 A	11-10-1990
		CA 2013396 A	05-10-1990
		JP 2291965 A	03-12-1990
		NZ 233109 A	23-12-1992
		JP 3206958 A	10-09-1991
US 5270165 A	14-12-1993	EP 0527823 A	24-02-1993
		US 5427931 A	27-06-1995
		WO 9116628 A	31-10-1991
		US 5213962 A	25-05-1993
WO 9834634 A	13-08-1998	US 5981471 A	09-11-1999
		AU 6277198 A	26-08-1998
		EP 0971723 A	19-01-2000
EP 0330725 A	06-09-1989	DE 3724581 A	02-02-1989
		JP 1124389 A	17-05-1989

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
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PCT

(10) International Publication Number
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(51) International Patent Classification[7]: C12Q 1/68

(21) International Application Number: PCT/EP00/08554

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1 September 2000 (01.09.2000)

(25) Filing Language: English

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00101921.5 1 February 2000 (01.02.2000) EP

(71) Applicant *(for all designated States except US)*: **EVOTEC NEUROSCIENCES GMBH** [DE/DE]; Schnackenburgallee 114, 22525 Hamburg (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **RICHARD, Giesbert** [DE/DE]; Universitäts Augenklinik, Eppendorf, Martinistrasse 52, 20246 Hamburg (DE). **NITSCH, Roger** [DE/CH]; Guggerstrasse 19, CH-8702 Zollikon (CH).

(74) Agents: **MEYERS, Hans-Wilhelm** et al.; P.O. Box 10 22 41, 50462 Cologne (DE).

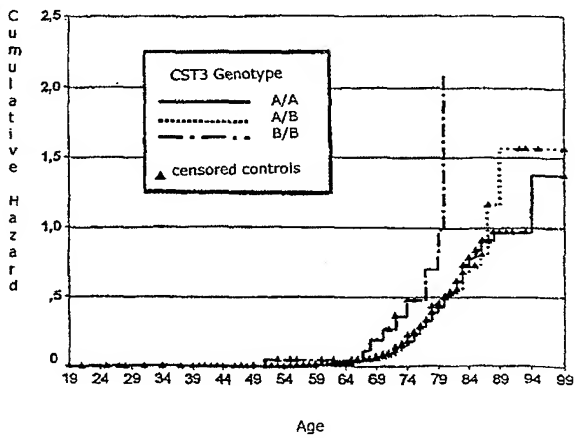
(81) Designated States *(national)*: JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS OF DIAGNOSING OR PROGNOSTICATING AGE-RELATED MACULAR DEGENERATION



(57) Abstract: A method for diagnosing or prognosing age-related macular degeneration in a subject, or determining whether a subject is at increased risk of developing age-related macular degeneration or monitoring the progression of age-related macular degeneration in a subject, comprising: determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, an amyloid protein, and a transcription product of a gene coding for an amyloid protein in a sample from said subject; and comparing said level, or said activity, or both said level and said activity, of at least one of said substances to a reference value representing a known disease or health status, thereby diagnosing or prognosing said age-related macular degeneration in said subject, or determining whether said subject is at increased risk of developing age-related macular degeneration or monitoring the progression of said age-related macular degeneration in said subject.

WO 01/16364 A2

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/16364 A2

(51) International Patent Classification[7]: C12Q 1/68

(21) International Application Number: PCT/EP00/08554

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(26) Publication Language: English

(30) Priority Data:
99117198.4 1 September 1999 (01.09.1999) EP
00101921.5 1 February 2000 (01.02.2000) EP

(71) Applicant *(for all designated States except US)*: **EVOTEC NEUROSCIENCES GMBH** [DE/DE]; Schnackenburgallee 114, 22525 Hamburg (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **RICHARD, Giesbert** [DE/DE]; Universitäts Augenklinik, Eppendorf, Martinistrasse 52, 20246 Hamburg (DE). **NITSCH, Roger** [DE/CH]; Guggerstrasse 19, CH-8702 Zollikon (CH).

(74) Agents: **MEYERS, Hans-Wilhelm** et al.; P.O. Box 10 22 41, 50462 Cologne (DE).

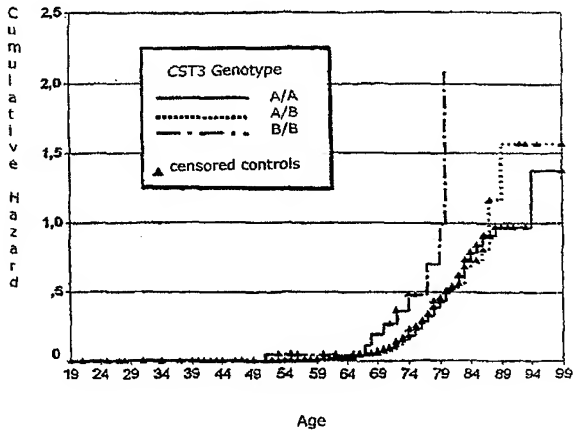
(81) Designated States *(national)*: JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS OF DIAGNOSING OR PROGNOSTICATING AGE-RELATED MACULAR DEGENERATION



(57) Abstract: A method for diagnosing or prognosing age-related macular degeneration in a subject, or determining whether a subject is at increased risk of developing age-related macular degeneration or monitoring the progression of age-related macular degeneration in a subject, comprising: determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, an amyloid protein, and a transcription product of a gene coding for an amyloid protein in a sample from said subject; and comparing said level, or said activity, or both said level and said activity, of at least one of said substances to a reference value representing a known disease or health status, thereby diagnosing or prognosing said age-related macular degeneration in said subject, or determining whether said subject is at increased risk of developing age-related macular degeneration or monitoring the progression of said age-related macular degeneration in said subject.

WO 01/16364 A2

Methods of diagnosing or prognosticating age-related macular degeneration

Age-related macular degeneration (AMD) is the most common geriatric eye disorder leading to blindness. Macular degeneration is responsible for visual handicap in what is estimated conservatively to be approximately 16 million individuals worldwide. Among the elderly, the overall prevalence is estimated between 5.7 % and 30 % depending on the definition of early AMD, and its differentiation from features of normal aging, a distinction that remains poorly understood (Ferris et al., Arch. Ophthalmol., 102: 1640 – 1642, 1984; Klein et al., Ophthalmology, 104: 7 – 21, 1997). Histopathologically, the hallmark of early neovascular AMD is accumulation of extracellular drusen and basal laminar deposit (abnormal material located between the plasma membrane and basal lamina of the retinal pigment epithelium) and basal linear deposit (material located between the basal lamina of the retinal pigment epithelium and the inner collageneous zone of Bruch's membrane). The end stage of AMD is characterised by a complete degeneration of the neurosensory retina and of the underlying retinal pigment epithelium in the macular area. Advanced stages of AMD can be subdivided into geographic atrophy and exudative AMD. Geographic atrophy is characterized by progressive atrophy of the retinal pigment epithelium. In exudative AMD the key phenomenon is the occurence of choroidal neovascularisation (CNV). Eyes with CNV have varying degrees of reduced visual acuity, depending on location, size, type and age of the neovascular lesion. The development of choroidal neovascular membranes can

be considered a late complication in the natural course of the disease possibly due to tissue disruption (Bruch's membrane) and decompensation of the underlying longstanding processes of AMD.

Many pathophysiological aspects as well as vascular and environmental risk factors are known to be associated with a progression of the disease, but little is known about the etiology of AMD itself as well as about the underlying processes of complications like the occurence of CNV.

Family, twin, segregation, and case-control studies suggest an involvement of genetic factors in the etiology of AMD. However, the extent of heritability, number of genes involved, and mechanisms underlying phenotypic heterogeneity are unknown. The search for genes related to AMD faces challenges: The onset is late in life, and there is usually only one generation available for studies. The parents of patients are often deceased, and the children are too young to manifest the disease. Generally, the heredity of late-onset diseases has been difficult to estimate because of the uncertainties of the diagnosis in previous generations and the inability to diagnose AMD among the children of an affected individual. Even in the absence of the ambiguities in the diagnosis of AMD in previous generations, the late onset of the condition itself, natural death rates, and small family sizes result in underestimation of genetic forms of AMD, and in overestimation of rates of sporadic disease. Moreover, the phenotypic variability is considerable, and it is conceivable that the currently used diagnostic entity of AMD in fact represents a spectrum of underlying conditions with various genetic and environmental factors involved. The search for genetic factors related to AMD has to address these challenges.

Contradictory results were reported for a possible role of *ApoE* polymorphisms in AMD. Some authors have reported a lower frequency of the ε4 allele in subgroups of AMD, while other reports do not confirm this association (Souied et al., Am. J. Ophthalmol., 125: 353 – 359, 1998; De La Paz et al., Invest Ophthalmol. Vis. Sci., 38(3): S796, Abstract nr 3695, 1997). The ε2 allele was

reported more frequent in AMD patients (Cruickshanks et al., Invest. Ophthalmol. Vis. Sci., 38(3): S471, Abstract nr 2187, 1997). For instance, also Klaver et al. (Am. J. Human Genet. 63: 200 – 206, 1998) have shown that the apoE gene (APOE) polymorphism is significantly associated with the risk for AMD. The APOE ε4 allele was associated with a decreased risk, and the ε2 allele was associated with a slightly increased risk of AMD. Their results suggest that APOE is a susceptibility gene for AMD.

Further, allelic variations in the *ABCR* gene were proposed to be associated with advanced atrophic AMD (Allikmets et al., Science, 277: 1805 – 1807, 1997), but again, other authors found no evidence to support this hypothesis (Stone et al., Nature Genet., 20: 328 – 329, 1998). Together, these studies illustrate the challenge to identify susceptibility genes in a most likely complex genetic disorder with the influence of unknown extents of environmental factors.

Experimental therapies for AMD have been suggested by Soubrane and Coscas (Wiedemann P., Kohen L. (eds): Macular and retinal diseases. Dev. Ophthalmol. Basel, Karger vol. 29, 77 – 84, 1997). The goal of therapies can be twofold: (i) prevention of the occurence of macular complications or (ii) treatment of the already arised macular complications (central geography atrophy or choroidal neovascularization). One approach for prevention includes antioxidant supplementation with contradictory results regarding the effectiveness. Laser photocoagulation has also been largely ineffective in preventing visual loss in the majority of patients. However, in the therapy of macular complications, laser photocoagulation remains the gold standard for the 15 % of well-defined choroidal new vessels (CNVs). For isolated occult CNVs and vascularized pigment epithelium detachments, no effective treatment is at the horizon. For geographic atrophy, the only hope is at present retinal pigment epithelium and photoreceptors transplantation.

As AMD is an important medical problem, there is a strong need for methods of diagnosing or prognosticating said disease in subjects as well as for methods of treatment. In addition, there is a strong need for identifying inherited risk factors that increase the susceptibility of getting AMD (susceptibility gene).

It was therefore an object of the present invention to provide methods of diagnosing or prognosticating age-related macular degeneration. Another object of the present invention was to provide methods of monitoring the progression of this disease and of evaluating a treatment for it. Still a further object of the present invention was to provide kits and agents suitable to be used in the aforementioned methods. Another object was to provide a method for identifying agents which affect age-related macular degeneration.

These objects have been solved by the methods, kits and agent according to the features of the independent claims. The sub-claims define preferred embodiments thereto.

The term „and/or" used in the present specification and in the claims implies that the phrases before and after this term are considered either as alternatives or as a combination. For instance, the wording „determination of a level and/or an activity" means that either only a level, or only an activity, or both a level and an activity are determined.

The term „gene" used in the present specification and in the claims comprises both coding regions (exons) as well as non-coding regions (e.g. non-coding regulatory elements such as a promotor or enhancers; introns; leader & trailer sequences).

In one aspect, the invention features a method for diagnosing or prognosticating age-related macular degeneration in a subject, or determining whether a subject is at increased risk of becoming diseased with age-related

macular degeneration. The method includes: determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, an amyloid protein, and a transcription product of a gene coding for said amyloid protein in a sample from said subject; and comparing said level, or said activity, or both said level and said activity, of at least one of said substances to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said age-related macular degeneration in said subject, or determining whether said subject is at increased risk of becoming diseased with age-related macular degeneration.

In a further aspect, the invention features a method of monitoring the progression of age-related macular degeneration in a subject. A level, an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, an amyloid protein, and a transcription product of a gene coding for an amyloid protein in a sample from said subject is determined. The level and/or activity of at least one of the aforementioned transcription and/or translation products or fragments thereof is compared to a reference value representing a known disease or health status, thereby monitoring the progression of age-related macular degeneration.

In still a further aspect, the invention features a method of evaluating a treatment for age-related macular degeneration, comprising: determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, an amyloid protein, and a transcription product of a gene coding for an amyloid protein in a sample obtained from a subject being treated for said age-related macular degeneration. The level

and/or activity of at least one of the aforementioned transcription and/or translation products or fragments thereof is compared to a reference value representing a known disease or health status, thereby evaluating the treatment of AMD.

By determining the level and/or activity of the Cystatin C mRNA, a translation product of a Cystatin gene, or a fragment of said translation product (e.g. Cystatin C itself) the activity of the Cystatin C gene can be assessed.

The human Cystatin C gene (*CST3*) maps to chromosome 20p11.2; it contains three exons. Four point mutations in the promoter region of the human Cystatin C gene have been detected by direct sequencing of polymerase chain reaction amplified D N A. The four base changes are all localized within a short segment of 85 base pairs. Three Cystatin C gene alleles could be defined with respect to these promoter mutations. Mendelian inheritance of the polymorphisms has been demonstrated in a study of Caucasian individuals showing frequencies of Cystatin C genotypes AA, BB, CC, AB, AC, and BC. The *Sst* II polymorphic site within the 5' flanking sequence is in linkage disequilibrium with a second *Sst* II polymorphism within exon 1 of the gene. An Ala/Thr variation in the coding region of the human Cystatin C gene has been detected as said *SSt* II polymorphism. Because of said linkage disequilibrium, the aforementioned polymorphisms in the *CST 3* gene result in the human haplotypes termed *CST 3 A* (nucleotides G, A, and G at positions -157, -72 and +73), and *CST 3 B* (nucleotides C, C, and A at these positions) (Balbin and Abrahamson, Hum. Genet. 81, 751 - 752, 1991; Balbin et al., Hum. Genet. 92, 206 - 207, 1993; Abrahamson et al., FEBS Lett. 216, 229 - 233, 1987; the contents of these publications are incorporated herein by reference). The open reading frame of *CST3* encodes a 120-residue protein with a molecular mass of 13.3 kDa and a pI of 8.75 (Abrahamson et al., J. Biol. Chem. 261, 11282 - 11289, 1986; Abrahamson et al., FEBS Lett. 216, 229 - 233, 1987; the contents of these publications are incorporated herein by reference). The mature molecule contains intramolecular disulfide bonds, it is

partially hydroxylated, no other common post-translational modifications were observed (Grubb and Löfberg, Proc. Natl. Acad. Sci., USA, 79, 3024 - 3027, 1982; Asgeirsson et al., Biochem. J., 329, 497 - 503, 1998; the contents of these publications are incorporated herein by reference). The production rate of Cystatin C is remarkably constant and its plasma concentration can therefore be used as a reliable measure of the glomerular filtration rate (Grubb, Clinical Nephrology, Vol. 38, Suppl. No.1, 20 - 27, 1992). Cystatin C is distributed extensively in the body fluids and is suspected of playing a role in extracellular functions, such as the modulation of inflammatory reactions. It is known to exist in cell types, such as astrocytes, macrophages, and choroid plexus cells. Cystatin C also is a quantitatively dominating cysteine protease inhibitor of cerebrospinal fluid (CSF) whose concentration is five times higher than that of plasma. It binds to and regulates proteolytic activities of cathepsins which have been associated with brain amyloid plaques in Alzheimer's disease, and implicated in the proteolytic processing of the amyloid precursor protein. It has been described that human Cystatin C undergoes dimerization before unfolding. Dimerization leads to a complete loss of its activity as a cysteine proteinase inhibitor (Ekiel et al., J. Mol. Biol. 271, 266 - 277, 1997; the contents of which are incorporated herein by reference). In addition to the termination of biological activity, dimerization and aggregation of Cystatin C is associated with brain amyloid formation in the Islandic form of hereditary cerebral hemorrhage with amyloidosis caused by an inherited mutation (L68Q) within the coding region of *CST3*.

Expression of the human Cystatin C gene leads to a primary transcript containing two intervening sequences. Splicing of this RNA results in a single mRNA species. Translation of the mRNA generates a primary translation product containing a leader sequence in front of the mature Cystatin C amino acid sequence. During secretion of the polypeptide across the membrane of the endoplasmatic reticulum, the leader sequence is cleaved off at the signal peptide cleavage site, giving rise to the secretory Cystatin C molecule. Variations of amino acid positions within the primary translation product,

particularly changes at or close to the signal peptide cleavage site, might influence the processing of the precursor protein. Unprocessed or incorrectly processed Cystatin C proteins might cause major irritations of cellular functions, e.g. processing and secretion, and disturb the balance of proteases and protease inhibitors in cells and tissues. This might lead to an increase in the production of amyloidogenic peptides, e.g. by cleavage of amyloid precursor protein (APP) into amyloid ß protein. Alternatively, the altered processing of the Cystatin C precursor might result in an increased tendency of the respective protein to aggregate and thereby enhance the aggregation of other amyloidogenic proteins and peptides, e.g. amyloid ß protein.

The role of amyloid proteins, in particular amyloid β protein, in neurodegenerative disorders has been extensively described in literature (see e.g. Harper J.D. and Lansbury P.T., Annu. Rev. Biochem., 66, 385 – 407, 1997). The family of Aβ variants is derived from the amyloid precursor protein (APP), a ubiquitous ca. 700-amino acid cell-surface protein. Two variants, Aβ1-40 and Aβ1-42, which differ by truncation at the carboxyl terminus are the predominant amyloid plaque proteins.

Preferred embodiments of the above mentioned methods for diagnosing or prognosticating AMD, or determining an increased risk of becoming diseased with AMD, or monitoring the progression of AMD, or evaluating a treatment of AMD are now disclosed in detail.

Preferably, the Cystatin C gene is a polymorphic variant of the wild-type gene. The presence of at least one B allele, in particular the B/B genotype indicates said subject is at increased risk of developing AMD or indicates diagnosis or prognosis of AMD.

It might be preferred that said subject has previously been determined to have one or more factors indicating that such subject is afflicted with AMD.

In a further preferred embodiment, Aβ1-40 and/or Aβ1-42 are determined as amyloid protein.

In preferred embodiments, the sample is taken from a body fluid, a tissue, or an organ – in particular the eye - of said subject. It is particularly preferred to take a sample from material located between the plasma membrane and basal lamina of the retinal pigment epithelium and/or from material located between the basal lamina of the retinal pigment epithelium and the inner collagenous zone of Bruch's membrane.

According to the present invention, a variation of the level of a translation product of a Cystatin C gene, a fragment thereof, a transcription product of a Cystatin C gene, an amyloid protein, and/or a transcription product of a gene coding for said amyloid protein in said sample from the subject relative to a reference value indicates a diagnosis, or prognosis, or increased risk of said age-related macular degeneration in said subject. As shown in Example 1, a significantly higher frequency of the CST3 BB genotype is observed in patients with exudative AMD. A specific feature associated with this genotype might be an abnormal level of the active form of Cystatin C in specific tissues and body fluids. In a further embodiment, a varied activity of Cystatin C or a translation product of a Cystatin C gene in a sample from a subject relative to a corresponding reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of AMD in said subject. The varied activity can be due to a polymorphism in the coding region of the Cystatin C gene, leading to a phenotype which differs from the wild-type phenotype. Varied levels of Cystatin C may be due to polymorphisms in the regulatory elements, in particular the promoter, of the Cystatin C gene.

In preferred embodiments, measurement of the level of transcription products of the Cystatin C gene and/or a gene coding for said amyloid protein is performed using Northern blots with probes specific for said genes. Quantitative PCR with primer combinations to amplify gene-specific sequences

from cDNA obtained by reverse transcription of RNA extracted from said sample of a subject can also be applied. These techniques are known to those of ordinary skill in the art (see e.g. Watson et al., Rekombinierte DNA, 2nd edition, Spektrum Akademischer Verlag GmbH, Heidelberg, 1993; Watson et al., Recombinant DNA, 2nd ed., W. H. Freeman and Company, 1992).

In preferred embodiments, said level/activity of a translation product of a Cystatin C gene, or fragments thereof - such as Cystatin C -, or said amyloid protein is detected using an immunoassay. These assays can e.g. measure the amount of binding between Cystatin C and an anti-Cystatin C antibody by the use of enzymatic, chromodynamic, radioactive, or luminescent labels which are attached to either the anti-Cystatin C antibody or a secondary antibody which binds the anti-Cystatin C antibody. In addition, other high affinity ligands including cathepsin derivatives may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow et al., Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

The antibody or ligand to be used should preferably specifically detect a translation product of a Cystatin C gene, or a fragment thereof (e. g. Cystatin C) or said amyloid protein. It is preferred that it does not substantially interact with any other protein present in said sample. It is particularly preferred to include specific antibodies or ligands which differentiate monomers from dimers or oligomers of Cystatin C. The detection of the monomeric form of Cystatin C may be more specific to age-related macular degeneration than measuring dimers or oligomers. Measures might be significantly improved with monomer-specific ELISAs.

Monoclonal antibodies capable of recognizing said translation products of the Cystatin C gene, or fragments thereof (e. g. Cystatin C), or said amyloid proteins can be prepared using methods known in the art (see e.g. Köhler and Milstein, Nature 256, 495 - 497 1975; Kozbor et al., Immunol. Today 4, 72,

1983; Cole et al., Monoclonal antibodies and cancer therapy, Alan R. Liss, Inc., pp 77 - 96, 1985; Marks et al., J. Biol. Chem., 16007 - 16010, 1992; the contents of which are incorporated herein by reference). Such monoclonal antibodies or fragments thereof can also be produced by alternative methods known to those of skill in the art of recombinant DNA technology (see e.g. Sastry et al, PNAS 86: 5728, 1989; Watson et al., Rekombinierte DNA, 2nd ed., Spektrum Akademischer Verlag GmbH, 1993; Watson et al, Recombinant DNA, 2nd ed., W. H. Freeman and Company, 1992; the contents of which are incorporated herein by reference). Monoclonal antibodies useful in the methods of the invention are preferably directed to an epitope of Cystatin C or said amyloid protein, such that the complex formed between the antibody and Cystatin C, or between the antibody and said amyloid protein, can be recognized in detection assays. The term "antibodies" encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, single chain antibodies as well as fragments thereof which specifically bind to a translation product of a Cystatin C gene, a fragment thereof (e.g. Cystatin C or its subfragments), or to an amyloid protein. It is particularly preferred to use specific antibodies that selectively detect Cystatin C monomers, dimers or oligomers, respectively. High-affinity ligands can be prepared by using derivatives of cathepsins which are the natural substrates of Cystatin C biological activity.

It is further preferred to determine e.g. the level of Cystatin C and its activity on basis of an enzymatic assay. As described above, Cystatin C is a cysteine protease inhibitor which binds to and regulates proteolytic activities of cathepsins. A suitable enzymatic assay can therefore be built upon the enzymatic activity of cathepsins indicating the absense or presence of different levels of Cystatin C in its active, monomeric form. It is preferred to use amyloid precursor protein (APP) as a substrate. The generation of A-beta peptides as educts can be measured.

The determination of the level or activity of a translation product of a Cystatin C gene, or a fragment thereof (e.g. Cystatin C), or an amyloid protein, can also be performed on basis of a binding assay. Suitable binding partners include cathepsins or fragments thereof, peptides, peptidomimetics, antibodies and other chemical probes which can specifically recognize the aforementioned substances. It is in general particularly preferred to determine Cystatin C in its monomeric form. Suitable binding partners for an amyloid protein assay include e.g. peptides, peptidomimetics, antibodies and other chemical probes.

If luminescent labels are used in any detection assay, it is preferred to use a confocal optical set-up. It is particularly preferred to conduct detection assays utilising fluorescence techniques known to the person skilled in the art, e. g. fluorescence correlation spectroscopy, FRET, fluorescence anisotropy measurements, fluorescent lifetime measurements, or fluorescence intensity distribution analysis.

In preferred embodiments, the reference value can be that of a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a translation product of a Cystatin C gene, a fragment thereof (e. g. Cystatin C), a transcription product of a Cystatin C gene, an amyloid protein, and a transcription product of a gene coding for said amyloid protein in a sample from a subject not suffering from age-related macular degeneration. The healthy subject can be of the same weight, age, and gender as the subject who is being diagnosed or prognosed for AMD, or for whom an increased risk of becoming diseased with AMD is determined. In some cases, it might be preferred to use a reference value from the subject which is diagnosed.

In preferred embodiments, the subject can be a human, an experimental animal, e. g. a rat or a mouse, a domestic animal, or a non-human primate, e.g. a monkey. The experimental animal can be an animal model for a disorder, e.g. a transgenic mouse with an AMD pathology.

In a preferred embodiment, the level, or the activity, or both said level and said activity, of at least one of said substances in a sample is determined at least twice, e.g. at two points which are weeks or months apart. The levels or activities at these two time points are compared in order to monitor the progression of AMD. It might further be preferred to compare a level, or an activity, or both said level and said activity of at least one substance which is selected from the group consisting of a translation product of a Cystatin C gene, a fragment thereof (e. g. Cystatin C or its subfragments), a transcription product of a Cystatin C gene, an amyloid protein and a transcription product of a gene coding for said amyloid protein in said sample with a level, an activity, or both said level and said activity, of at least one of said substances in a series of samples taken from said subject over a period of time. In further preferred embodiments, said subject receives a treatment prior to one or more of said sample gatherings. Preferably, said level, or said activity, or said level and said activity, is determined before and after a treatment for AMD is administered to said subject. This procedure is suitable for evaluating a treatment of AMD.

In another aspect, the invention features, a method of diagnosing or prognosticating age-related macular degeneration in a subject, or determining whether a subject is at increased risk of becoming diseased with age-related macular degeneration. The method includes: determining the presence or absense of a mutation or polymorphism in a Cystatin C gene in a sample from said subject, thereby diagnosing, or prognosticating, or determining an increased risk of AMD in said subject. The term „gene" as used in the present specification comprises coding as well as non-coding regions, e.g. non-coding regulatory elements such as a promotor or enhancer sequences. Such a mutation can e.g. be a substitution, deletion or addition of at least one base. Such mutations may result in "mis-sense" information or in "non-sense" information associated with a termination codon or a frame shift. Polymorphisms and allele variations occur more frequently in the general

population but induce in principle the identical genetic alterations. In case of polymorphisms or mutations in the coding region of the gene, the translation product or processed peptides/proteins thereof may have an amino acid sequence which differs from that of the translation products or processed peptides/proteins thereof in their wild-type form. In case of a polymorphic phenotype, usually a control subject with a wild-type Cystatin C gene will be chosen. In this control subject, one will therefore determine only wild-type Cystatin C. However, polymorphisms might also be extant in regions preceding and/or following the coding region (leader and trailer) or in intervening sequences (introns) between individual coding segments (exons). Such mutations or polymorphisms may be found within the promoter region, an example of which is the *Sst* II polymorphic site in the promoter region of the human Cystatin C gene (*CST 3*).

It is preferred to determine the presence of a B allele in the Cystatin C gene. The human Cystatin C gene, called *CST3*, has been sequenced and its A and B alleles were also described (Balbin et al., Biol. Chem. Hoppe-Seyler, Vol. 373, 471 - 476, 1992; Abrahamson et al., Biochem. J. 268, 287 - 294, 1990; Abrahamson et al., Hum. Genet. 82, 223 - 226, 1989; the contents of these publications are incorporated herein by reference). The presence of at least one B allele in said Cystatin C gene indicates said subject and potentially its descendants are at increased risk of developing age-related macular degeneration. In particular, homozygous *CST 3* B/B subjects are at increased risk of developing AMD. The data shown in Example 1 indicate an association of *CST3* B/B genotype with AMD. In a further preferred embodiment, a disease-predisposing mutation identifiable by linkage analysis of the mutations in said B allele might also indicate that the subject under study is at increased risk of developing AMD.

Determining the presence or absense of a mutation or polymorphism in a Cystatin C gene in a sample from said subject may comprise determining a partial nucleotide sequence of the DNA from said subject, said partial

nucleotide sequence indicating the presence or absence of said mutation or polymorphism. It may further be preferred to perform a polymerase chain reaction with the DNA from said subject and subsequent restriction analysis to determine the presence or absence of said mutation or polymorphism. Suitable primers can be used for amplifying the promoter region as well as the coding sequence of exon 1 of the human Cystatin C gene in order to subsequently analyse the *Sst* II polymorphic sites herein. For the determination of mutations or polymorphisms in the Cystatin C gene, it is preferred to use DNA from body cells, in particular white blood cells.

In another preferred embodiment, the method further includes: determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a translation product of a Cystatin C gene, a fragment of said translation product (e.g. Cystatin C), a transcription product of a Cystatin C gene, an amyloid protein, and a transcription product of a gene coding for said amyloid protein in a sample from said subject; and comparing said level, or said activity, or both said level and said activity, of at least one of said substances to a reference value. A variation (in particular an increase) of a level of a translation product of a Cystatin C gene, a fragment thereof (e. g. Cystatin C or its subfragments), or a transcription product of a Cystatin C gene in a sample from a patient relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of AMD in the patient. In a further embodiment, a varied activity of Cystatin C or a varied processing of the Cystatin C precursor proteins (i.e. the translation products of the Cystatin C gene) in a sample from the patient relative to a reference value representing a known health status also indicates a diagnosis, or prognosis, or increased risk of AMD. The varied activity/processing can be due to a polymorphism in the coding region of the Cystatin C gene, leading to a phenotype which differs from the wild-type phenotype.

In another aspect, the invention features, a kit and its use for diagnosis, or prognosis of age-related macular degeneration, or for determination of increased risk of developing AMD, or for monitoring a progression of age-related macular degeneration in a subject, or for monitoring success or failure of a therapeutic treatment of said subject.

Said kit comprises at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product a Cystatin C gene, (ii) reagents that selectively detect a translation product of a Cystatin C gene and/or a processed or fragmented peptide of the translation product, (iii) reagents that selectively detect a mutation or polymorphism in a Cystatin C gene, and (iii) reagents that selectively detect a transcription product and/or a translation product of a gene coding for an amyloid protein. Preferably, it further comprises instructions for use. Processing of the translation product might lead to the natural Cystatin C, however also mis-processing might take place, leading to fragments of the translation product which differ from the normally occurring Cystatin C. The use preferably comprises (i) detecting a level, or an activity, or both said level and said activity, of said Cystatin C, or of said transcription/translation products of said Cystatin C gene, or of said amyloid protein, or of said transcription products of a gene coding for an amyloid protein, in a sample from said subject; and/or (ii) detecting a presence or absence of mutations or polymorphisms in said Cystatin C gene in a sample from said subject. A varied level, or activity, or both said level and said activity, of at least one of the aforementioned substances, compared to a reference value representing a known health status indicates a diagnosis, or prognosis, or an increased risk of developing AMD. Also the presence of a mutation or polymorphism in said Cystatin C gene indicates a diagnosis, or prognosis, or an increased risk of developing age-related macular degeneration.

It is preferred that said at least one reagent and said instructions are packaged in a single container. In preferred embodiments, said reference

value is that of a level, or an activity, or both said level and said activity, of at least one substance which is selected from the above mentioned group in a sample from a subject not suffering from said AMD. The healthy subject can be of the same weight, age, and gender as the subject who is being diagnosed, or prognosed for AMD, or for whom an increased risk of developing AMD is determined. In some cases, it might be preferred to use a reference value of the subject which is to be diagnosed. Said kit suitable for commercial manufacture and sale can still further include appropriate standards, positive and negative controls.

The kit preferably comprises reagents that selectively detect the presence of at least one B allele in said Cystatin C gene, in particular the presence of the B/B genotype. This indicates a diagnosis, or prognosis, or an increased risk of age-related macular degeneration. In order to exclude a false positive diagnosis, it should be remarked that a mutation or polymorphism in the Cystatin C gene in the codon for leucine at position 68 which abolishes an *Alu*I restriction site is not indicative for age-related macular degeneration, but for hereditary Cystatin C amyloid angiopathy.

Determining the presence or absense of a mutation or polymorphism in a Cystatin C gene in a sample from said subject may comprise determining a partial nucleotide sequence of the DNA from said subject, said partial nucleotide sequence indicating the presence or absence of said mutation or polymorphism. It may further be preferred to perform a polymerase chain reaction with the DNA from said subject and subsequent restriction analysis to determine the presence or absence of said mutation. Therefore, in a preferred embodiment, said kit comprises primers for amplifying at least parts of the promoter region and/or of the coding region of a Cystatin C gene in order to subsequently analyze the *Sst* II (or isoenzyme) polymorphic sites herein.

In preferred embodiments, the constituents of said kit allow for measurement of the level of transcription products of the Cystatin C gene, or of a gene

coding for an amyloid protein. This measurement is e.g. performed using Northern blots with probes specific for said genes. Quantitative PCR with primer combinations to amplify gene-specific sequences from cDNA obtained by reverse transcription of RNA extracted from body cells of a subject can also be applied. These techniques are known to those of ordinary skill in the art (see e.g. Watson et al., Rekombinierte DNA, 2nd edition, Spektrum Akademischer Verlag GmbH, Heidelberg, 1993; Watson et al., Recombinant DNA, 2nd ed., W. H. Freeman and Company, 1992).

For the determination of mutations or polymorphisms in the Cystatin C gene, it is preferred to use DNA from body cells including fibroblasts and white blood cells. For the other analyses, it is particularly preferred to use samples taken from the eye, as explained above.

In preferred embodiments, the constituents of said kit allow for the detection of said level and/or activity of said above mentioned substances using an immunoassay. These assays can e.g. measure the amount of binding between Cystatin C and an anti-Cystatin C antibody by the use of enzymatic, chromodynamic, radioactive, or luminescent labels which are attached to either the anti-Cystatin C antibody or a secondary antibody which binds the anti-Cystatin C antibody. In addition, other high affinity ligands including cathepsin derivatives may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow et al., Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

The antibody or ligand to be used should preferably specifically detect a translation product of a Cystatin C gene, a fragment of said translation product (e. g. Cystatin C), or an amyloid protein. It is preferred that the antibody or ligand does not substantially interact with any other protein present in said sample. It is particularly preferred to include in said kit specific antibodies or ligands which differentiate monomers from dimers or oligomers of Cystatin C.

The detection of the monomeric form of Cystatin C may be more specific to AMD than measuring dimers or oligomers. Measures might be significantly improved with monomer-specific ELISAs.

Monoclonal antibodies capable of recognizing a translation product of a Cystatin C gene, a processed peptide thereof (e. g. Cystatin C), or an amyloid protein can be prepared using methods known in the art (see e.g. Köhler and Milstein, Nature 256, 495 - 497 1975; Kozbor et al., Immunol. Today 4, 72, 1983; Cole et al., Monoclonal antibodies and cancer therapy, Alan R. Liss, Inc., pp 77 - 96, 1985; Marks et al., J. Biol. Chem., 16007 - 16010, 1992; the contents of which are incorporated herein by reference). Such monoclonal antibodies or fragments thereof can also be produced by alternative methods known to those of skill in the art of recombinant DNA technology (see e.g. Sastry et al, PNAS 86: 5728, 1989; Watson et al., Rekombinierte DNA, 2nd ed., Spektrum Akademischer Verlag GmbH, 1993; Watson et al., Recombinant DNA, 2nd ed., W. H. Freeman and Company, 1992; the contents of which are incorporated herein by reference). Monoclonal antibodies useful in the kit of the invention are preferably directed to an epitope of Cystatin C or an amyloid protein, such that the complex formed between the antibody and Cystatin C, or between the antibody and said amyloid protein, can be recognized in detection assays. The term "antibodies" encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, single chain antibodies as well as fragments thereof which specifically bind to a translation product of a Cystatin C gene, a processed peptide thereof such as Cystatin C, or to an amyloid protein. It is particularly preferred to use specific antibodies that selectively detect Cystatin C monomers, dimers or oligomers, respectively. High-affinity ligands can be prepared by using derivatives of cathepsins which are the natural substrates of Cystatin C biological activity.

It is further preferred that the kit comprises constituents which allow to determine the level of Cystatin C and its activity on basis of an enzymatic assay. As described above, Cystatin C is a cysteine protease inhibitor which

binds to and regulates proteolytic activities of cathepsins. A suitable enzymatic assay can therefore be built upon the enzymatic activity of cathepsins indicating the absense or presence of different levels of Cystatin C in its active, monomeric form. It is preferred to use amyloid precursor protein (APP) as a substrate. The generation of A-beta peptides as educts can be measured.

The determination of the level or activity of a translation product of a Cystatin C gene, a fragment thereof (e. g. Cystatin C), or an amyloid protein, can also be performed on basis of a binding assay. Suitable binding partners include cathepsins or fragments thereof, peptides, peptidomimetics, antibodies and other chemical probes which can specifically recognize said translation product or Cystatin C. It is in general particularly preferred to determine Cystatin C in its monomeric form. Suitable binding partners for an amyloid protein assay include e.g. peptides, peptidomimetics, antibodies and other chemical probes. The aforementioned binding partners are preferably constituents of a kit according to the present invention.

In another aspect, the invention features a method of treating or preventing AMD in a subject comprising administering to said subject in a therapeutically effective amount an agent or agents which directly or indirectly modulates a biological activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a Cystatin C gene, a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a processed/fragmented peptide thereof (e. g. Cystatin C), a gene coding for an amyloid protein, a transcription product of a gene coding for an amyloid protein, and an amyloid protein.

It is preferred that said agent or agents reduce a biological activity, or level, or both said activity and level, of at least one of the mentioned substances.

In preferred embodiments, said agents bind or inhibit Cystatin C, as e.g. cathepsin derivatives or Cystatin C analoga. In further preferred

embodiments, said agents inhibit the formation of macular plaques, in particular drusen or amyloid plaques, in said subject's eyes.

In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or agents.

In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogeneous cellular gene expression by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, Acc. Chem. Res. 26, 274 - 278, 1993; Mulligan, Science 260, 926 - 931, 1993; the contents of which are incorporated herein by reference) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane perturbation by chemical (solvents, detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, electric shocks).

In particular, the invention features a method of treating or preventing age-related macular degeneration by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells (see e.g. Gillespie, DN & P 5(7), 389 - 395, 1992; Agrawal, Tibtech 13, 197 - 199, 1995; Crooke, Bio/Technology 10, 882 - 886, 1992; the contents of which are incorporated herein by reference). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that act as enzymes, destroying RNA that carries the message of disease has also been described (see e.g. Barinaga, Science, 262, 1512 - 1514, 1993; the contents of which are incorporated herein by reference). In preferred embodiments, the subject to be treated is a human, and therapeutic antisense nucleic acids or

derivatives thereof are directed against the human Cystatin C gene *CST-3*, or transcription products of *CST-3*. Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particles, or the above described techniques. Strategies for administering targeted therapeutic oligodeoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, Tibtech, 10, 281 - 287, 1992; the contents of which are incorporated herein by reference). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed *ex vivo* with a recombinant gene that directs the synthesis of an RNA that is complementary to a region of the target nucleic acid. Therapeutically use of intracellularly expressed antisense RNA is procedurally similar to gene therapy.

In preferred embodiments, the method comprises grafting donor cells into the eye of said subject, said subject or donor cells preferably treated so as to minimise or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phospate transfection, liposomal mediated transfection, etc.

In preferred embodiments, said agent is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector, in particular a retroviral vector.

In preferred embodiments, the therapeutic nucleic acid or protein reduces amyloid formation by interacting with a Cystatin C gene, its transcription products, a translation product of a Cystatin C gene, or a processed peptide thereof such as Cystatin C. Said amyloid is e.g. β-amyloid derived by proteolytic processing of the amyloid precursor protein (APP) known in Alzheimer's disease.

In preferred embodiments, the subject can be a human, an experimental animal, e.g. a rat or a mouse, a domestic animal, or a non-human primate, e.g. a monkey. The experimental animal can be an animal model for a disorder, e.g. a transgenic mouse with an AMD pathology.

In another aspect, the invention features a modulator of an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a Cystatin C gene, a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a processed/fragmented peptide thereof (e. g. Cystatin C), a gene coding for an amyloid protein, a transcription product of a gene coding for an amyloid protein, and an amyloid protein. This modulator or agent might directly or indirectly affect a biological activity, or level, or both said activity and level, of at least one of the aforementioned substances.

It is preferred that said modulator(s) reduce(s) a biological activity, or level, or both said activity and level, of at least one substance which is selected from the above mentioned substances. In preferred embodiments, the agent is a therapeutic nucleic acid or protein which reduces amyloid formation by interacting with a Cystatin C gene, its transcription/translation products, or Cystatin C. It might also interact with an amyloidic peptide, preferably β-amyloid derivable by proteolytic processing of the amyloid precursor protein (APP) known in Alzheimer's disease.

In a further aspect, the invention features a medicament comprising a modulator of biological activity, or level, or both said activity and level, of at least one substance which is mentioned above. It is preferred that said modulator reduces a biological activity, or level, or both said activity and level, of at least one of said substances.

In another aspect, the invention features an agent/modulator which directly or indirectly affects a biological activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a Cystatin C gene, a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a processed/fragmented translation product such as e. g. Cystatin C, a gene coding for an amyloid protein, a transcription product of a gene coding for an amyloid protein, and an amyloid protein, for treating or preventing age-related macular degeneration. It is preferred that said agent(s) reduce(s) a biological activity, or level, or both said activity and level, of at least one of said substances. In preferred embodiments, the agent is a therapeutic nucleic acid or protein which reduces amyloid formation by interacting with a Cystatin C gene, its transcription products, or a un/processed translation product of a Cystatin C gene. If the modulator is capable of interacting with an amyloidic protein, this amyloid is preferably β-amyloid derivable by proteolytic processing of the amyloid precursor protein (APP) known in Alzheimer's disease.

In a further aspect, the invention features the use of a modulator of a biological activity, or level, or both said activity and level, of at least one substance which is selected from the group consitsing of a Cystatin C gene, a transcription product of a Cystatin C gene, an (un)processed translation product of a Cystatin C gene, a gene coding for an amyloid protein, a transcription product of a gene coding for an amyloid protein, and an amyloid protein, for a preparation of a medicament for treating or preventing age-related macular degeneration. It is preferred that said modulator(s) reduce(s) a biological activity, or level, or both said activity and level, of at least one of

said substances. In preferred embodiments, the agent is a therapeutic nucleic acid or protein which reduces amyloid formation by interacting with a Cystatin C gene, its transcription products, or the processed translation product Cystatin C. It is preferred that it interacts with β-amyloid which is derivable by proteolytic processing of the amyloid precursor protein (APP) known in Alzheimer's disease.

In another aspect, the invention features a method for identifying an agent that affects age-related macular degeneration, comprising the steps of:

- providing a sample containing at least one substance which is selected from the group consisting of a Cystatin C gene, a transcription product of a Cystatin C gene, an (un)processed translation product of a Cystatin C gene, a gene coding for an amyloid protein, a transcription product of a gene coding for an amyloid protein, and an amyloid protein;
- contacting said sample with at least one agent; and
- comparing an activity, or level, or both said activity and level, of at least one of said substances before and after said contacting.

It is preferred that said agent reduces an activity, or level, or both said activity and level, of at least one of said substances.

In preferred embodiments, measurement of the level of transcription products of a Cystatin C gene, or of a gene coding for an amyloid protein, is performed using Northern blots with probes specific for said genes. Quantitative PCR with primer combinations to amplify gene-specific sequences from cDNA obtained by reverse transcription of RNA extracted from a subject can also be applied. These techniques are known to those of ordinary skill in the art (see e.g. Watson et al., Rekombinierte DNA, 2nd edition, Spektrum Akademischer Verlag GmbH, Heidelberg, 1993; Watson et al., Recombinant DNA, 2nd ed., W. H. Freeman and Company, 1992).

In preferred embodiments, said level or activity of a translation product of a Cystatin C gene, of a processed peptide thereof such as Cystatin C, or of an amyloid protein, is detected using an immunoassay. These assays can e.g. measure the amount of binding between Cystatin C and an anti-Cystatin C antibody by the use of enzymatic, chromodynamic, radioactive, or luminescent labels which are attached to either the anti-Cystatin C antibody or a secondary antibody which binds the anti-Cystatin C antibody. In addition, other high affinity ligands including cathepsin derivatives may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow et al., Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

The antibody or ligand to be used should preferably specifically detect Cystatin C or an amyloid protein. It is preferred that it does not substantially interact with any other protein present in said sample. It is particularly preferred to include specific antibodies or ligands which differentiate monomers from dimers or oligomers of Cystatin C.

Monoclonal antibodies capable of recognising a translation product of a Cystatin C gene, a processed peptide thereof (e. g. Cystatin C), or an amyloid protein, can be prepared using methods known in the art (see e.g. Köhler and Milstein, Nature 256, 495 - 497 1975; Kozbor et al., Immunol. Today 4, 72, 1983; Cole et al., Monoclonal antibodies and cancer therapy, Alan R. Liss, Inc., pp 77 - 96, 1985; Marks et al., J. Biol. Chem., 16007 - 16010, 1992; the contents of which are incorporated herein by reference). Such monoclonal antibodies or fragments thereof can also be produced by alternative methods known to those of skill in the art of recombinant DNA technology (see e.g. Sastry et al, PNAS 86: 5728, 1989; Watson et al., Rekombinierte DNA, 2nd ed., Spektrum Akademischer Verlag GmbH, 1993; Watson et al., Recombinant DNA, 2 nd., W. H. Freeman and Company, 1992; the contents of which are incorporated herein by reference). Monoclonal antibodies useful in the

methods of the invention are preferably directed to an epitope of Cystatin C or an amyloid protein, such that the complex formed between the antibody and Cystatin C, or between the antibody and said amyloid protein, can be recognised in detection assays. The term "antibodies" encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, single chain antibodies as well as fragments thereof which specifically bind to a translation product of a Cystatin C gene, a fragment thereof (e. g. Cystatin C), or said amyloid protein. It is particularly preferred to use specific antibodies that selectively detect Cystatin C monomers, dimers or oligomers, respectively. High-affinity ligands can be prepared by using derivatives of cathepsins which are the natural substrates of Cystatin C biological activity.

It is further preferred to determine the level of an (un)processsed translation product of a Cystatin C gene, e. g. Cystatin C, and its activity on basis of an enzymatic assay. As described above, Cystatin C is a cysteine protease inhibitor which binds to and regulates proteolytic activities of cathepsins. A suitable enzymatic assay can therefore be built upon the enzymatic activity of cathepsins indicating the absense or presence of different levels of Cystatin C in its active, monomeric form. It is preferred to use amyloid precursor protein (APP) as a substrate. The generation of A-beta peptides, in particular A-beta 40 and A-beta 42 peptides, as educts can be measured.

The determination of the level or activity of an unprocessed or processed translation product of a Cystatin C gene, or an amyloid protein, can also be performed on basis of a binding assay. Suitable binding partners include cathepsins or fragments thereof, peptides, peptidomimetics, antibodies and other chemical probes which can specifically recognise Cystatin C, or a precursor or fragment thereof. It is in general particularly preferred to determine Cystatin C in its monomeric form. Suitable binding partners for an amyloid protein assay include e.g. peptides, peptidomimetics, antibodies and other chemical probes.

If luminescent labels are used in any detection assay, it is preferred to use a confocal optical set-up. Preferably fluorescence techniques can be used, e.g. fluorescence correlation spectroscopy, fluorescence intensity distribution analysis, fluorescence lifetime analysis, FRET, or fluorescence anisotropy measurements.

The accompanying figures illustrate the present invention. Other features and advantages of the invention will be apparent from the following detailed description of the examples, and from the claims.

Figure 1 depicts a schematic illustration of the human Cystatin C gene with the exons numbered and shown as filled boxes as well as the three different alleles (A, B and C) with their respective nucleotide sequences given around the mutations in the promotor region. Nucleotide numbering for the base substitutions relates to the start site for Cystatin C translation (+1 - +3 equals initiator methionine codon). The polymorphic *Sst* II and *Dde* I sites are underlined, and expected lengths of DNA fragments after respective cleavage are indicated. Please note that the *Sst* II polymorphic site within the 5' flanking sequence is in linkage disequilibrium with a second *Sst* II polymorphism within exon 1 of the gene (not shown). An Ala/Thr variation in the coding region of the human Cystatin C gene has been detected as said *SSt* II polymorphism. Because of said linkage disequilibrium, the aforementioned polymorphisms in the *CST 3* gene result in the human haplotypes termed *CST 3 A* (nucleotides G, A, and G at positions -157, -72 and +73), and *CST 3 B* (nucleotides C, C, and A at these positions).

Figure 2 depicts the possible roles of Cystatin C and the *Aspergillus japonicus* cysteine proteinase inhibitor E-64 in the amyloid precursor protein (APP) processing and generation of the amyloid β peptide (A_β).

Figure 3 depicts a Kaplan-Meier Hazard Function: Cumulative Hazard dependent on age and *CST3* genotype. For further details see EXAMPLE 2.

EXAMPLE 1

Patients and Methods

Between February and October 1998, 167 patients with the clinical diagnosis of exudative AMD of all types, classic CNV, occult CNV and pigment epithelial detachment were recruited at the University Eye Hospital Hamburg-Eppendorf, Germany. 268 unrelated age-matched control subjects were randomly selected to represent a sample of the general population, and therefore can be expected to develop AMD at the population rate (Table 1). The possibility of selecting elderly control subjects without signs of AMD was not chosen because this would require the differentiation of features associated with normal aging from early AMD. This distinction is difficult and its theoretical basis is weak. Informed consent was obtained from all patients and controls. This study was conducted according to the tenets of the Declaration of Helsinki and was approved by our institutional human experimentation committee. Inclusion criteria for patients were: Diagnosis of uni- or bilateral neovascular AMD by using fluorescein angiography and absence of other retinal dystrophies or diseases that may be associated with the development of CNV.

A comprehensive ophthalmologic examination which included visual acuity measurement, fundus examination and fluorescein angiography was done in patients. Age at presentation and gender were recorded for both patients and control subjects. No age of onset of AMD was recorded for patients because due to the lingering course of the early forms often no definite date can be given.

Fluorescein angiographic photographs were evaluated according to the guidelines of the international classification and grading system of the international ARM epidemiological study group by two independent graders. Grading results were subsequently reviewed by another independent grader

(The international ARM Epidemiological Study Group. An international classification and grading system for age-related maculopathy and age-related macular degeneration. Surv. Ophthalmol. 39(5): 367 - 374, 1995). Inclusion criteria for the patient group were the presence of any form of neovascular maculopathy secondary to age-related macular degeneration including classic CNV, occult CNV, pigment epithelial detachment, or a combination of any of the above in one or both eyes. If only one eye was affected by neovascular AMD, the other eye had to present with phenomena of early AMD including more than 5 hard drusen, soft drusen, pigmentary abnormalities, or with advanced atrophic AMD (geographic atrophy) to ensure that AMD was the cause of neovascular maculopathy. In cases of disagreement between the two initial graders the conclusive grading was done by a the reviewing grader. To avoid bias, all graders were blinded with respect to the results of the genotype analyses.

Blood was collected from peripheral veins into EDTA tubes from patients and control subjects. Genomic DNA was extracted from isolated leukocytes using a standard salt precipitation technique and its concentration was determined spectrophotometrically. The PCR product was generated using the primers 024, TGGGAGGGACGAGGCGTTCC and 1206R, TCCATGGGGCCTCCCACCAG. A 10 µl polymerase chain reaction was performed, containing 0.4 µl of suspended genomic DNA, 500 nM of each forward and reverse primer, 1.5 mM magnesium chloride, 1 µl 10x buffer, 200 µM deoxyribonucleoside triphosphate, 0.4 U Taq polymerase (Gibco, Gaithersburg, MD), 0.5 µl of 5% dimethyl sulfoxide and 6.7µl H_2O. For a negative control, no DNA was added to the PCR reaction contents. The reactions were denaturated for 45 seconds at 95 °C, then DNA was amplified for 13 cycles (15 seconds at 95 °C, 30 seconds at 68 °C reduced by 1 °C per cycle and 30 seconds at 72 °C) followed by 23 cycles (15 seconds at 95 °C, 30 seconds at 55°C, 30 seconds at 72 °C) then followed by a final 5-minute extension at 72 °C. The PCR product is a 318 bp DNA fragment, 1 µl of each PCR product was electrophoresed on a 2.5 % agarose gel, stained with ethidium bromide, and visualized under UV

illumination. After each PCR reaction, the remaining 9 µl of PCR product was used for enzyme digestion with 10 units *Sac II* (MBI Fermentas, Vilnius, Lithuania) in 10 mM $MgCl_2$, 10 mM Tris-HCl, 0.2 mg bovine serum albumin (BSA) at pH 7.5 in 9 µl purified water at 37°C overnight. Enzyme digestion revealed fragment sizes of 41, 51 and 226 bp from the A-allele and 127 and 191 bp from the B-allele, respectively. Haplotypes were confirmed by direct sequencing of PCR products from individuals with the genotypes AA, AB and BB. The digestion products were electrophoresed and visualized in the same manner as described for PCR products. They can easily be distinguished on electrophoresis.

Statistical analyses were done with SPSS (SPSS Inc., Chicago, IL). The non-parametric Pearson chi-square test was used to compare the *CST3*-BB bearers versus nonbearers consisting of the genotypes AA and AB (df=1). Kaplan-Meier survival analysis was performed on data obtained from 167 patients and 268 controls. P-values less than 0.05 were considered as statistically significant. Means and standard deviations were calculated for age of patients and controls.

Results

The analysis of demographic data revealed that age at presentation was similar for the male and female subgroup of AMD patients and control subjects (Table 1). Both genders were separately age-matched since the number of male and female subjects among patients and controls was unequally distributed ($p<0.0001$). The genotype frequencies of *CST3* haplotypes were in Hardy-Weinberg equilibrium in both patients and controls.

Simultaneous genotyping of three polymorphic *SacII* restriction sites in the 5`-region of *CST3* covered by a single PCR fragment revealed a strong linkage disequilibrium between all three polymorphic *SacII* sites. The observed

haplotypes were defined by either concomitant *SacII* restriction, one at 80 bp upstream of the transcription start sites and one in the penultimate codon of the sequence that encodes the signal peptide (allele A), or by exclusive cleavage downstream of the transcription start sites (allele B).

The frequency of homozygous haplotype *CST3* BB genotype differed significantly between patients with exudative AMD and control subjects (p=0.0228; Table 2). The frequency of this genotype was 0.066 for the entire patients group compared with 0.022 for control subjects overall. When subgroups for both genders were formed the frequency of the BB genotype was 0.113 in male patients and 0.024 in male controls. There was a statistically significant difference of the BB genotype distribution in the male subgroup (p=0.0119; Fisher's exact test: p=0.0201; Table 3). The frequency of the BB genotype in the female subgroup was 0.044 in patients versus 0.021 in controls and failed to differ statistically (p=0.303;) among groups (Table 4).

The effect of the BB genotype on the occurrence of exudative AMD was estimated using a odds ratio estimate (OR) in case control studies. This OR was 3.079 (95% confidence interval [CI] 1.116 to 8.490) for the entire groups of patients and controls. When the male subgroups of cases and controls were tested separately, the effect was stronger with a OR of 5.276 (95% CI 1.267 to 21.961). For the female subgroup the OR was calculated to be 2.11 (95% CI 0.493 to 9.024). Data are shown in Table 5.

Kaplan-Meier survival analysis was performed on the data obtained from patients and control subjects. The average disease-free survival time was 83 years (95 % CI = 82 - 85; S.E.=1) in the pooled *CST3* AA or AB subjects and 75 years (95 % C I= 71 - 79; S.E.=2) in *CST3* BB subjects reaching statistical significance (Mantel-Cox Log rank = 9.00; df = 1; p = 0.0027).

Table 1: Patients with AMD and control subjects

	Patients	Controls
Sex (No.[%])		
Female	114 (42.5)	141 (57.5)
Male	53 (31.7)	127 (68.3)
mean age at presentation (yrs.±SD)		
Female	75.25 ± 7.63	75.30 ± 7.89
Male	73.57 ± 7.41	73.66 ± 6.63

Table 2: *CST3* genotype distribution

CST3 genotype	Patients (n=167)		Controls (n=268)	
	n	%	n	%
AA or AB	156	93.4	262	97.8
BB	11	6.6	6	2.2

$\chi^2 = 5.18$; p = 0.0228; df = 1

Table 3: *CST3* genotype distribution among males

CST3 genotype	Patients (n=53)		Controls (n=127)	
	n	%	n	%
AA or AB	47	88.7	124	97.6
BB	6	11.3	3	2.4

$\chi^2 = 6.318$; p = 0.0119; df = 1; Fisher´s exact test: p = 0.0202

Table 4: *CST3* genotype distribution among females

CST3 genotype	Patients (n=114)		Controls (n=141)	
	n	%	n	%
AA or AB	109	95.6	138	97.9
BB	5	4.4	3	2.1

$\chi^2 = 1.056$; p = 0.303; df = 1

Table 5: Odds ratios for exudative AMD with *CST3* genotype BB dependent on gender

subgroup	Odds ratio	95% CI
all patients	3.079	1.117-8.49
female	2.11	0.493-9.024
male	5.276	1.267-21.961

EXAMPLE 2

Patients and methods

Patients (n = 167, age range 51 - 94 years, 114 females and 53 males, with mean ages at presentation (SD) of 75.3 (7.6) and 73.6 (7.4) years) of Example 1 were studied. The 167 patients with advanced exudative AMD represented a subset of 200 AMD patients that included patients with geographic atrophy (n = 20) or early AMD (n = 7) in one or both eyes and did not have CNV. They were therefore excluded from this study. Thus, 88 % of all AMD patients had advanced exudative AMD in at least one eye, a typical rate for our tertiary care hospital. This reflects the fact that patients with choroidal neovascularization are much more likely to suffer severe vision loss and are therefore cared for by a specialised retinal centre.

Also 517 unrelated Caucasian control subjects (age range 19 - 99 years), 283 females and 234 males with mean age of 69.5 (12.7) and 66.3 (11.5) years, respectively. In order to allow the assessment of possible regional or ethnical differences in allele frequencies, the controls consisted of an international collection of adult volunteers originating from Germany (n = 235), Switzerland

(n = 164), Italy (n = 56), and USA (n = 62). The controls were not examined for ophthalomologic disorders and were expected to develop AMD at the population rate, and there were no exclusion criteria with respect to macular appearance in the control group.

Genomic D N A was isolated from peripheral blood leukocytes using a standard salt precipitation technique. PCR products (318 bp) from genomic D N A were generated by using primer 024, TGGGAGGGACGAGGCGTTCC (Balbin et al., Hum. Genet. 87(6): 751 - 752, 1991) and 1206R, TCCATGGGGCCTCCCACCAG. A 10 µl polymerase chain reaction was performed, containing 0.4 µl of suspended genomic D N A, 500 nM of each forward and reverse primer, 1.5 mM magnesium chloride, 1 µl 10 x buffer, 200 µM deoxyribonucleoside triphosphate, 0.4 units *Taq* D N A polymerase (Gibco, Gaithersburg, MD), 0.5 µl of 5 % dimethyl sulfoxide and 6.7 µl H_2O. The thermoprofile was 95 °C 45 sec., 13 x [95 °C 15 sec., 68 °C 30 sec –1 °C per cycle, 72 °C 30 sec], 23 x [95 °C 15 sec., 55 °C 30 sec, 72 °C 30 sec], and 72°C 5 min. Three polymorphic *KspI* restriction sites in the 5'-region of *CST3* were covered by the 318 pb PCR fragment. Through a strong linkage disequilibrium between the three polymorphisms only two haplotypes were observed. The haplotypes are defined by either concomitant *KspI* restriction endonuclease cleavage both 80 bp upstream of the mRNA transcription start site and in the penultimate codon of the signal peptide (haplotype A), or by an exclusive cleavage downstream of the transcription start site (haplotype B). Haplotypes were confirmed by direct sequencing of the PCR products from individuals with the genotypes A/A, A/B, and B/B. Restriction digestion of the PCR product with *KspI* (MBI Fermentas, Vilnius, Lithuania) at 37 °C overnight revealed fragment sizes of 41 / 226 / 51 bp (homozygote haplotype A), or 127 / 191 bp (homozygote haplotype B), or all five fragments in A/B heterozygotes. The digestion products were electrophoresed on a 2.5 % agarose gel, stained with ethidium bromide, and visualised under UV light.

All statistical association analyses were done with SPSS, version 8.0 (SPSS Inc., Chicago, IL). P values less than 0.05 were considered significant. Statistical analyses of deviations from Hardy Weinberg equilibrium (HWE) were done by

$\chi^2 = (X / X_{obs.} - X / X_{exp.})^2$, where $X / X_{obs.}$ is the observed genotype count of the respective *CST3* genotypes (A/A, A/B, or B/B) and $X / X_{exp.}$ is the respective genotype count expected under HWE which is calculated based on the frequencies (F_A and F_B) of the observed allelic variants (here: A and B) at a given locus: $F_A + F_B = 1$, therefore $(F_A + F_B)^2 = 1 = (F_A)^2 + (F_B)^2 + 2\ F_AF_B$. Note: $(F_A)^2 = A / A_{exp.}$, $(F_B)^2 = B / B_{exp.}$, $2\ F_AF_B = A / B_{exp.}$.

There was no significant difference in allele frequency of haplotype B (F_B) in the control groups of the four centers in Germany, Switzerland, Italy and USA with F_B of 0.18, 0.20, 0.21, and 0.21, respectively ($p = 0.22$, $DF = 3$). The similar F_B between the German controls ($F_B = 0.181$) and those pooled from the other three centers with a mean $F_B = 0.184$ ($p = 0.88$, $DF = 1$) suggested widespread population similarity of F_B and allowed to pool all controls.

Table 6 shows the genotype counts of all patients and controls. None of the genotype counts significantly deviated from those expected under Hardy Weinberg equilibrium (HWE). There was a significant difference in genotype counts between patients and controls ($\chi^2 = 7.16$, exact $p = 0.028$, $DF = 2$; two sided Fisher exact test: $p = 0.037$). The strongest difference between patients and controls was observed in the B/B homozygotes with 6.6 % and 2.3 %, respectively, suggesting an odds ratio (OR) for AMD in association with *CST3* B/B of 2.97 (95 % CI 1.28; 6.86). The respective proportion of A/B heterozygotes was almost identical in patients and controls. The difference in F_B between patients and controls with $F_B = 0.23$ and 0.18, respectively, failed to reach statistical significance ($\chi^2 = 2.83$, $p = 0.093$, $DF = 1$) and may be explained by the higher proportion of B/B homozygotes in the patients. In accordance with this, there were slightly less ($B/B_{obs.} = 11$) than expected under HWE ($B/B_{exp.} = 17.3$) B/B homozygotes in the controls ($\chi^2 = 1.62$) and

more than expected ($B/B_{obs.}$ = 12 vs. $B/B_{exp.}$ = 8.4, χ^2 = 0.81) B/B homozygotes in the patients ($\Sigma\chi^2$ = 2.43, n.s.).

Logistic regression analysis entering *CST3* genotype (B/B vs. A/A or A/B), gender, and age revealed the highest coefficient (B) for *CST3* (B = 1.26, p = 0.005), and lower but significant B's for gnedr (B = 0.41, p = 0.038) and age (B = 0.06, p < 0.0001). Therefore the association between B/B homozygosity and AMD were reanalyzed both in separately age-matched males (53 patients, 138 controls) and females (114 patients, 211 controls). The results shown in Table 7 suggest a stronger association of B/B with AMD in males than in females.

In both males and females there was a significant effect of *CST3* B/B on disease-free survival analyzed by Kaplan-Meier analysis. In males with genotypes A/A or A/B the mean disease free survival time was 86 yrs (SE 2; 95 % CI 82; 89) which was 74 yrs (SE 4; 95 % CI 67; 81) in B/B homozygotes (Log Rank p = 0.041). The mean disease free survival time in pooled males and females with genotypes A/A or A/B was 85 yrs (SE 1; 95 % CI 83; 86) and 76 yrs (SE 2; 95 % CI: 72; 79) in B/B homozygotes (Log Rank p = 0.0006). The graphic plot of the hazard function of the pooled survival analysis is shown in Figure 3. The Log Rank statistics were very similar (p= 0.0005) if a means disease onset of 3 yrs prior to clinical presentation was assumed. The oldest control subject homozygous B/B was 75 yrs, and 137 of 517 controls (26.5 %) were older than 75 yrs. The oldest patient homozygous B/B was 85, whereas only 10 of 167 patients (6 %) were older than 85.

It was decided not to choose an AMD-free population, because the distinction between early AMD and normal aging is not well defined. This study design may indeed suffer from reduced statistical power, because the control group may well include subjects with AMD as well as subjects who could manifest the disease later in life as can be expected from the normal age-dependent prevalence of AMD.

Our genotypic association data, the absence of a significant difference in allele frequencies between patients and controls, and the survival analyses show an increased susceptibility for exudative AMD in *CST3* B/B homozygotes. Therefore the *CST3* haplotype B may be a recessive risk allele, significantly contributing to disease risk in up to 6.6 % of German AMD patients.

Table 6: *CST3* genotype counts and frequencies in patients with AMD and control subjects

	CST3 genotype counts and frequencies (%)			
	N	**A/A**	**A/B**	**B/B**
Patients	167	103 (61.7)	53 (31.7)	11 (6.6)
- Female	114	69 (60.5)	40 (35.1)	5 (4.4)
- Male	53	34 (64.2)	13 (24.5)	6 (11.3)
Controls	517	340 (65.8)	165 (31.9)	12 (2.3)
- Age matched, females	211	150 (71.1)	56 (26.5)	5 (2.4)
- Age Matched, males	138	89 (64.5)	46 (33.3)	3 (2.2)

Table 7: Influence of gender and age matching of controls on odds ratio (OR) for exudative AMD (167 patients; 114 female, 53 male) in association with *CST3* genotype B/B

	OR	95 % CI	p*
All 167 patients and 517 controls	2.967	1.284 – 6.857	0.013
females, controls age matched	1.890	0.535 – 6.670	0.329
males, controls age matched	5.745	1.328 – 23.888	0.015

* Two sided Fisher exact test

Claims

1. A method for diagnosing or prognosticating age-related macular degeneration in a subject, or determining whether a subject is at increased risk of developing age-related macular degeneration, comprising:

determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, an amyloid protein, and a transcription product of a gene coding for an amyloid protein in a sample from said subject;

and

comparing said level, or said activity, or both said level and said activity, of at least one of said substances to a reference value representing a known disease or health status,

thereby diagnosing or prognosticating said age-related macular degeneration in said subject, or determining whether said subject is at increased risk of developing age-related macular degeneration.

2. A method of monitoring the progression of age-related macular degeneration in a subject, comprising:

determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, an amyloid protein, and a transcription product of a gene coding for an amyloid protein in a sample from said subject;

and

comparing said level, or said activity, or both said level and said activity, of at least one of said substances to a reference value representing a known disease or health status,
thereby monitoring the progression of said age-related macular degeneration in said subject.

3. A method of evaluating a treatment for age-related macular degeneration, comprising:
determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, an amyloid protein, and a transcription product of a gene coding for an amyloid protein, in a sample obtained from a subject being treated for said age-related macular degeneration;
and
comparing said level, or said activity, or both said level and said activity, of at least one of said substances to a reference value representing a known disease or health status,
thereby evaluating said treatment for said age-related macular degeneration.

4. The method according to at least one of claims 1 to 3, wherein said sample is taken from a body fluid, a tissue, or an organ, in particular an eye, of said subject.

5. The method according to claim 4, wherein said sample is taken from material located between the plasma membrane and basal lamina of the retinal pigment epithelium.

6. The method according to claim 4, wherein said sample is taken from material located between the basal lamina of the retinal pigment epithelium and the inner collagenous zone of Bruch's membrane.

7. The method according to at least one of claims 1 to 6, wherein a variation of said level of Cystatin C or a transcription product of a Cystatin C gene in said sample from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said age-related macular degeneration in said subject.

8. The method according to at least one of claims 1 to 7, wherein a varied activity of Cystatin C in said sample from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said age-related macular degeneration in said subject.

9. The method according to at least one of claims 1 to 8, wherein said Cystatin C gene is a polymorphic variant of the Cystatin C wild-type gene.

10. The method according to claim 9, wherein the presence of at least one B allele indicates said subject is at increased risk of developing age-related macular degeneration or indicates a diagnosis or prognosis of age-related macular degeneration.

11. The method according to claim 10, wherein the presence of the B/B genotype indicates said subject is at increased risk of developing age-related macular degeneration or indicates a diagnosis or prognosis of age-related macular degeneration.

12. The method according to at least one of claims 1 to 11, wherein said subject is a human.

13. The method according to at least one of claims 1 to 12, wherein said Cystatin C is determined in its monomeric form.

14. The method according to at least one of claims 1 to 13, wherein at least one of said substances is detected using an immunoassay, an enzyme activity assay and/or a binding assay.

15. The method according to at least one of claims 1 to 14, wherein said reference value is that of a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, an amyloid protein, and a transcription product of a gene coding for an amyloid protein in a sample from a subject not suffering from said age-related macular degeneration.

16. The method according to at least one of claims 1 to 15, further comprising comparing a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, an amyloid protein, a transcription product of a gene coding for an amyloid protein in said sample with a level, an activity, or both said level level and said activity, of at least one of said substances in a series of samples taken from said subject over a period of time.

17. The method according to at least one of claims 1 to 16, wherein said subject receives a treatment prior to one or more of said sample gatherings.

18. The method according to claim 17, wherein said level, or said activity, or both said level and said activity, in said samples is determined, before and after said treatment is administered to said subject.

19. A method of diagnosing or prognosticating age-related macular degeneration in a subject, or determining whether a subject is at increased risk of developing age-related macular degeneration comprising:
determining a presence or absence of a mutation or polymorphism in a Cystatin C gene in a sample from said subject,
thereby diagnosing or prognosticating age-related macular degeneration in said subject, or determining whether said subject is at increased risk of developing age-related macular degeneration.

20. The method of claim 19, wherein the presence or absence of at least one B allele is determined.

21. The method of claim 20, wherein the presence of at least one B allele, in particular the presence of the B/B genotype, indicates said subject is at increased risk of developing age-related macular degeneration or indicates a diagnosis or prognosis of age-related macular degeneration.

22. The method of at least one of claims 19 to 21, further comprising:
determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, an amyloid protein, and a transcription product of a gene coding for an amyloid protein, in a sample from said subject;
and

comparing said level, or said activity, or both said level and said activity, of at least one of said substances to a reference value representing a known disease or health status.

23. The method according to claim 22, wherein a variation of said level of Cystatin C or a transcription product of a Cystatin C gene in said sample from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said age-related macular degeneration in said subject.

24. The method according to claim 22 or 23, wherein a varied activity of Cystatin C in said sample from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said age-related macular degeneration in said subject.

25. Use of a kit for diagnosis or prognosis of age-related macular degeneration, or for determination of increased risk of developing age-related macular degeneration, or for monitoring progression of age-related macular degeneration in said subject, or for monitoring success or failure of a therapeutic treatment of said subject, said kit comprising at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product and/or a translation product of a Cystatin C gene, (ii) reagents that selectively detect a fragment of a translation product of a Cystatin C gene, (iii) reagents that selectively detect a mutation or polymorphism in a Cystatin C gene, and (iv) reagents that selectively detect a transcription product and/or a translation product of a gene coding for an amyloid protein.

26. The use according to claim 25 wherein said reagents selectively detect a polymorphic variant of the wild-type Cystatin C gene.

27. The use according to claim 26 wherein said reagents selectively detect a B allele of the Cystatin C gene.

28. The use according to at least one of claims 25 to 27 for working the methods according to claims 1 to 24.

29. A method of treating or preventing age-related macular degeneration in a subject comprising administering to said subject in a therapeutically effective amount an agent or agents which modulate(s) an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a Cystatin C gene, a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, a gene coding for an amyloid protein, a transcription product of a gene coding for an amyloid protein, and an amyloid protein.

30. The method according to claim 29 wherein said agent(s) modulate the activity and/or level of (i) a polymorphic variant of the wild-type Cystatin C gene, and/or (ii) a transcription product of (i), and/or (iii) a fragmented or unfragmented translation product of (i),

31. The method according to claim 29 or 30, wherein said agents are cathepsin derivatives or Cystatin C analoga.

32. The method according to at least one of claims 29 to 31, wherein per se known methods of gene therapy and/or antisense nucleic acid technology are applied to administer said agent(s).

33. The method according to at least one of claims 29 to 32 comprising grafting donor cells into the eye of said subject, said subject or donor cells preferably treated so as to minimise or reduce graft rejection,

wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent(s).

34. A modulator of an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a Cystatin C gene, a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, a gene coding for an amyloid protein, a transcription product of a gene coding for an amyloid protein, and an amyloid protein.

35. A medicament comprising a modulator according to claim 34.

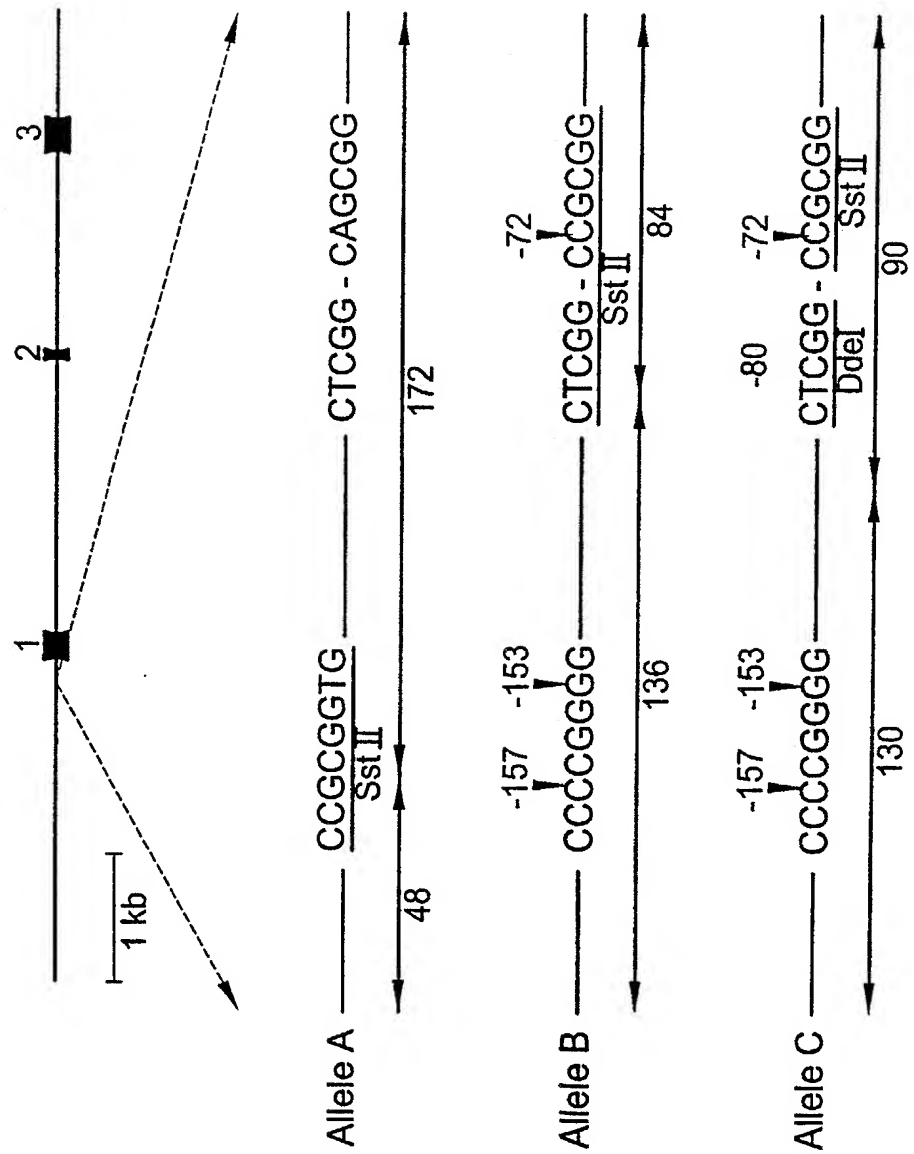
36. A modulator of an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a Cystatin C gene, a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, a gene coding for an amyloid protein, a transcription product of a gene coding for an amyloid protein, and an amyloid protein, for treating or preventing age-related macular degeneration.

37. The modulator of claims 34 or 36, wherein the modulator is capable of modulating a polymorphic variant of the wild-type Cystatin C gene, in particular a B allele.

38. Use of a modulator of an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a Cystatin C gene, a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, a gene coding for an amyloid protein, a transcription product of a gene coding for an amyloid protein, an amyloid protein, for a preparation of a medicament for treating or preventing age-related macular degeneration.

39. A method for identifying pharmaceutical modulators of age-related macular degeneration, comprising the steps of:
    - providing a sample containing at least one substance which is selected from the group consisting of a Cystatin C gene, a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, a gene coding for an amyloid protein, a transcription product of a gene coding for an amyloid protein, and an amyloid protein;
    - contacting said sample with at least one agent; and
    - comparing an activity, or level, or both said activity and level, of at least one of said substances before and after said contacting.

40. The method according to claim 39, wherein comparing an activity of Cystatin C is performed by using amyloid precursor protein as a substrate and a generation of A-beta peptides as a read-out.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Sst II
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-72
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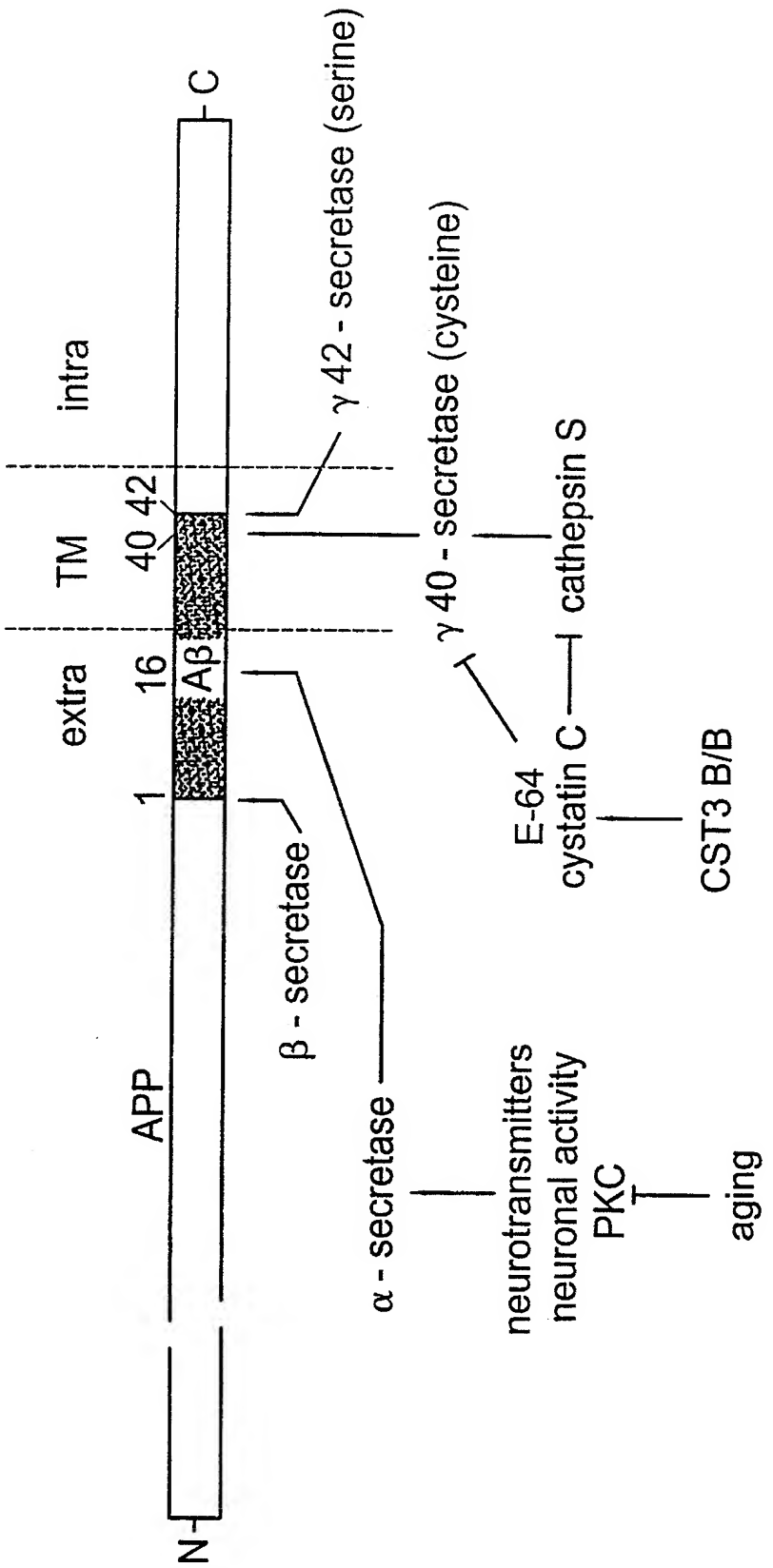
Ddel
Sst II
130
90

FIG.1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γ 40 - secretase (cysteine)
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extra
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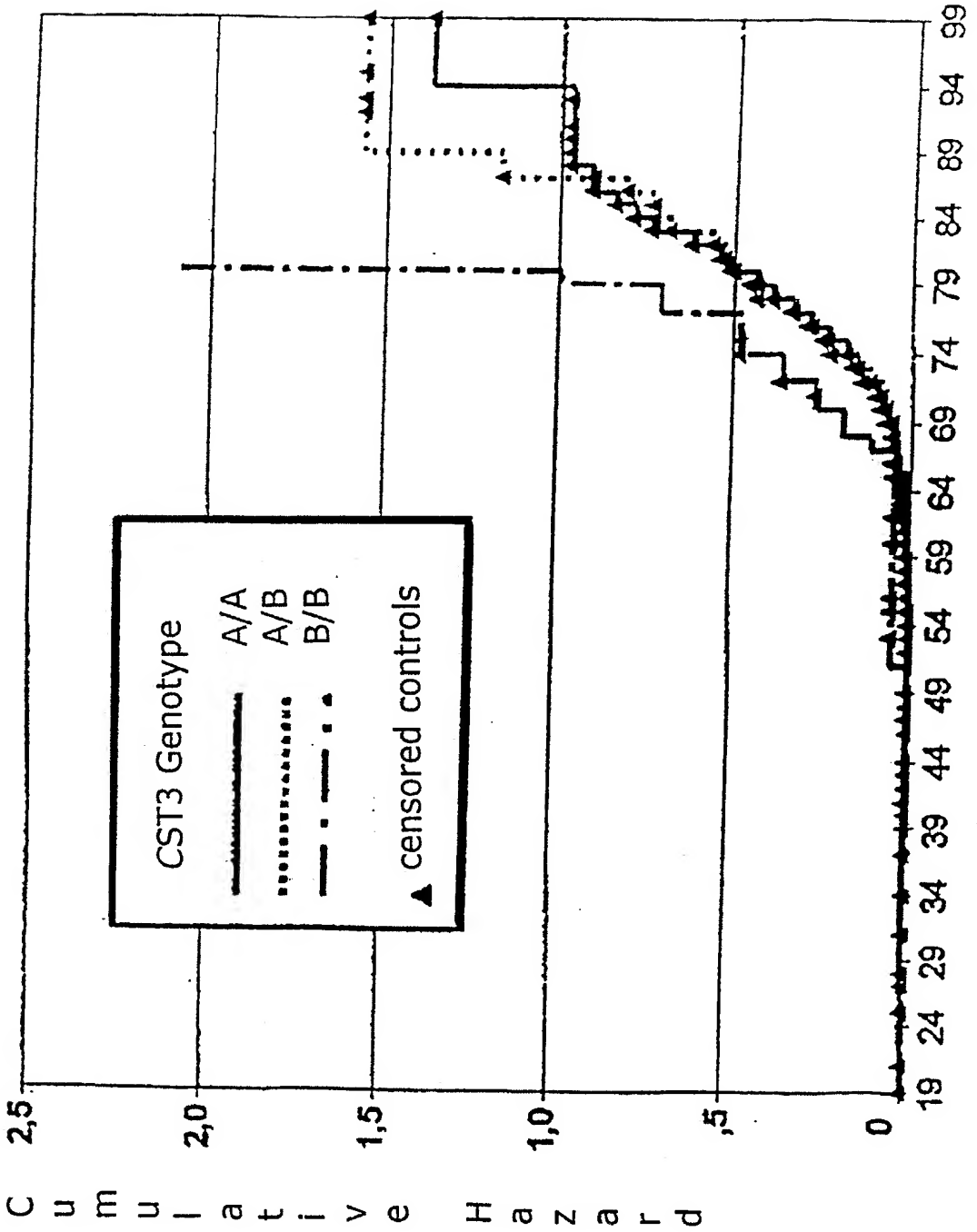
cystatin C
CST3 B/B
APP
α - secretase
neurotransmitters
neuronal activity
PKC
aging
N

FIG.2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2,0
1,5
1,0
,5
0
19 24 29 34 39 44 49 54 59 64 69 74 79 84 89 94 99
Age
CST3 Genotype
A/A
A/B
B/B
censored controls

Fig. 3

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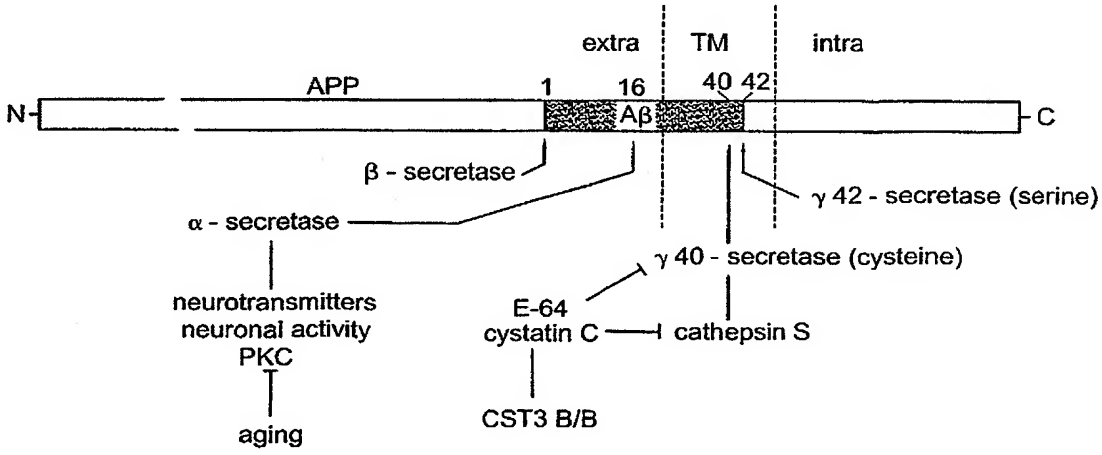
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS OF DIAGNOSING OR PROGNOSTICATING AGE-RELATED MACULAR DEGENERATION



(57) Abstract: A method for diagnosing or prognosing age-related macular degeneration in a subject, or determining whether a subject is at increased risk of developing age-related macular degeneration or monitoring the progression of age-related macular degeneration in a subject, comprising: determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of a transcription product of a Cystatin C gene, a translation product of a Cystatin C gene, a fragment of said translation product, an amyloid protein, and a transcription product of a gene coding for an amyloid protein in a sample from said subject; and comparing said level, or said activity, or both said level and said activity, of at least one of said substances to a reference value representing a known disease or health status, thereby diagnosing or prognosing said age-related macular degeneration in said subject, or determining whether said subject is at increased risk of developing age-related macular degeneration or monitoring the progression of said age-related macular degeneration in said subject.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/08554

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 C07K14/47 C07K14/81 C07K16/18 C07K16/38 C12N9/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, PAJ, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 231 000 A (MAJOCHA RON ET AL) 27 July 1993 (1993-07-27) column 2, line 6-36 column 4, line 45-68 claims 1-4 ---	1-33
A	EP 0 391 714 A (BRIGHAM & WOMENS HOSPITAL) 10 October 1990 (1990-10-10) page 4, line 48 - line 58 claims 17-23; example 1 ---	1-33
A	US 5 270 165 A (CUNNINGHAM DENNIS D ET AL) 14 December 1993 (1993-12-14) column 3, line 55 -column 4, line 7; claims 23,25 --- -/--	1-33

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 2001	12. 06. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Goetz, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/08554

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ABRAHAMSON M ET AL: "MOLECULAR CLONING AND SEQUENCE ANALYSIS OF CDNA CODING FOR THE PRECURSOR OF THE HUMAN CYSTEINE PROTEINASE INHIBITOR CYSTATIN C" FEBS LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 216, no. 2, 1 June 1987 (1987-06-01), pages 229-233, XP002038720 ISSN: 0014-5793 abstract page 229, right-hand column, last paragraph -page 230, left-hand column, paragraph 1; figure 2 ---	1-33
A	WO 98 34634 A (ENTREMED INC) 13 August 1998 (1998-08-13) page 5, line 1 -page 6, line 4 page 14, line 19 -page 15, line 6 ---	1-33
A	EP 0 330 725 A (GRUENENTHAL CHEMIE) 6 September 1989 (1989-09-06) page 4, line 53 -page 5, line 13; example 3 -----	1-33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/08554

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 29 - 33 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-33

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1 - 33

Methods relating to the diagnosis, the monitoring, the treatment or the evaluation of such treatment of age-related macular degeneration, as well as a the use of a kit for the diagnosis or prognosis of increased risk of age-related macular degeneration, said methods and kit being based on the determination of activity/level/mutations/polymorphisms of Cystatin C, its transcription/translation products and/or amyloid protein.

2. Claims: 34 - 40

Modulators which affect the activity and/or level of Cystatin C, its transcription/translation products and/or amyloid protein, method for identifying such modulator, medicament comprising said modulator and first/second medical use thereof.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PCT/EP 00/08554

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